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ENTOMON

Vol. 50 *June 2025* *No. 2*

Contents

Articles

Page

https://doi.org/10.33307/entomon.v50i2.1454 123
Ultra-structural description of *Haemaphysalis* species on goats in Kerala, south India
Kajal T Annasaheb, Bindu Lakshmanan, Amrutha Anand and Indu V Raj

https://doi.org/10.33307/entomon.v50i2.1456 131
Assessing the impact of foliar insecticides on yellow stem borer *Scirpophaga incertulas* and rice yield enhancement
B.N. Balaji, Pandit, L. Vijaykumar and B. Shivanna

https://doi.org/10.33307/entomon.v50i2.1457
Foraging interaction of stingless bee *Tetragonula travancorica* with flowers in ornamental plants 137
C.L. Anchu, V. Vijayasree , R.L. Abhijith and A. Sheena

https://doi.org/10.33307/entomon.v50i2.1458 145
In Silico biological evaluation of propolis for modulating Ghrelin, Leptin and CYP17A1 gene pathways in Polycystic Ovary Syndrome
Ashmitha Elangovan, N. Aarthi and P.B. Harathi

hhttps://doi.org/10.33307/entomon.v50i2.1459 153
Description of a new species of genus *Chloroplaga* Hampson (Lepidoptera, Nolidae) from India
Harkanwal Singh

https://doi.org/10.33307/entomon.v50i2.1460 157
Efficacy of botanicals and synthetic acaricides against *Aceria noxia* Flechtmann & Tassi, 2020 in vegetable amaranth, *Amaranthus tricolor* L.
K.P. Krishna Keerthi, K.M. Sreekumar, B. Ramesha, C. Vidya and P.K. Sajeesh

https://doi.org/10.33307/entomon.v50i2.1461 163
Effectiveness of plant essential oil nanoemulsions in controlling, *Leptocorisa oratoria* (Hemiptera, Alydidae) in lab and field conditions
M. Amrutha and Vimala K John

https://doi.org/10.33307/entomon.v50i2.1464 175
A new black fly species of subgenus *Gomphostilbia* Enderlein, 1921 (Simuliidae, Diptera) from Eastern Ghats, India
S. Vijayan and S. Anbalagan

https://doi.org/10.33307/entomon.v50i2.1465 183
Microbial analysis of hive stored pollen of Indian honey bee, *Apis cerana indica* (Fabricius) (Hymenoptera, Apidae)
V. Abhishek, Mani Chellappan, M.T. Ranjith, K. Surendragopal and Seeja Thomachan

https://doi.org/10.33307/entomon.v50i2.1469 191
A new species of *Zographetus* Watson, 1893 (Lepidoptera, Hesperiidae, Hesperiinae) from the Western Ghats, Peninsular India
Kalesh Sadasivan, Baiju Kochunarayanan, Sujitha Prabhakaran Chandrika, Toms Augustine, Muhamed Jafer Palot, Jebine Jose, Sojan Munnar and Vinayan Padmanabhan Nair

https://doi.org/10.33307/entomon.v50i2.1470 203
A new species of *Lyriothemis* Brauer, 1868 (Odonata, Libellulidae) from the Western Ghats of Peninsular India
Kalesh Sadasivan, Jebine Jose, Muhamed Jafer Palot, Shanas Sudheer, Toms Augustine, Baiju Kochunarayanan, Vinayan Padmanabhan Nair and Maya George

Short Communications

https://doi.org/10.33307/entomon.v50i2.1471 215
An unusual new record of *Pseudoathyreus orientalis* (Laporte, 1840) (Coleoptera, Bolboceratidae, Athyreinae), an eremian species from the Deccan Peninsula, India and designation of its neotype
Aparna Sureshchandra Kalawate

https://doi.org/10.33307/entomon.v50i2.1472 219
First record of the genus *Lemuriana* Distant, 1905 (Hemiptera, Cicadidae, Lamotialnini) from Kerala, India
A. Anjana and K.T. Teji

https://doi.org/10.33307/entomon.v50i2.1473 225
Evaluation of food consumption of *Zygogramma bicolorata* Pallister 1953, on *Parthenium hysterophorus* in Dehradun, Uttarakhand, India
Yashika Negi, Veena Maurya, Madhu Thapliyal and Ashish Uniyal

https://doi.org/10.33307/entomon.v50i2.1474 229
New distribution of the myrmicine arboreal ant, *Paratopula intermedia* Sheela and Narendran (Formicidae, Myrmicinae) from south India
Thresiamma Varghese

https://doi.org/10.33307/entomon.v50i2.1475 231
Record of *Ulomimus indicus* Bates, 1873 (Coleoptera, Tenebrionidae) from south India
T.K. Viswanath, V.D. Hegde and K.M. Remia

https://doi.org/10.33307/entomon.v50i2.1476 235
Leptocorisa Latreille (Alydidae) species abundance and distribution in paddy fields of Thrissur, Kerala, India
M. Amrutha and Vimala K John

https://doi.org/10.33307/entomon.v50i2.1477 239
Report on the rediscovery of *Thacona ferreopicta* (Hampson, 1900) (Lepidoptera, Scranciidae) with the first description of female genitalia from India
M.P. Unnikrishnan, Ryan A. St Laurent and Sapna Jacob

https://doi.org/10.33307/entomon.v50i2.1478 243
New records of egg parasitoids (Hymenoptera, Chalcidoidea, Trichogrammatidae) from Kashmir Valley, Jammu and Kashmir, India
Rabiya Irshad, Mohd Yousuf, Mohsin Ikram, Syed Faizan Anayat and Tahmeena Maqsood

https://doi.org/10.33307/entomon.v50i2.1479 247
Obituary - Dr. K.S.S. NAIR
Dr. George Mathew

Author Index 251

https://doi.org/10.33307/entomon.v50i2.1454
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Ultra-structural description of *Haemaphysalis* species on goats in Kerala, south India

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ABSTRACT: Ticks and tick-borne diseases are a major threat to goat farming in hot humid tropical regions of the world. The parasite species and vectors are significantly influenced by topographic and climatic variables. In the context of high prevalence of theileriosis in goats being reported from Kerala, south India, the ixodid tick fauna of the region has to be mapped. Adult ticks collected from 500 naturally infested goats were identified based on gross morphological and scanning electron microscopic studies. Majority of the ticks on goats belonged to *Haemaphysalis* genus (99.1%) followed by *Rhipicephalus.* Scanning electron microscopic features of *Haemaphysalis intermedia* (Warburton and Nuttall, 1909*)*, *H. bispinosa* (Neumann, 1897*)*, *H. kutchensis* (Hoogstraal & Trapido, 196) and *H. megalaimae* (Rajagopalan, 1963) were studied. *H. kutchensis* and *H. megalaimae* are reported for the first time from south India. This study emphasises the need to revisit the tick surveillance on different animal species of the state to provides an insight into the possible outbreaks of fatal tick-borne pathogens in the state.
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KEY WORDS: Ticks, *Haemaphysalis*, *H. kutchensis, Rhipicephalus,* ultrastructure, new report

INTRODUCTION

Ticks are haematophagous ectoparasites that cause significant economic losses to small ruminant farming through both their direct biting effects and the transmission of diseases. A substantial proportion (94.36%) of the world's goat population resides in Asia and Africa, highlighting the critical economic role of these small ruminants for impoverished communities in arid, semiarid, hilly, and remote tribal regions (Hegde, 2020). Goat farming in humid tropics faces numerous challenges, with tick and tick-borne diseases warranting particular attention. Ticks such as *Haemaphysalis bispinosa*, *Rhipicephalus sanguineus* and *R. microplus* have been identified as potential vectors for *Babesia motasi*, *B. ovis* and *Theileria ovis* infections in goats in India, Bangladesh and Pakistan (Ghosh *et al.,* 2007). Previous attempts to identify tick species on domestic animals in Kerala, revealed a high prevalence of *Haemaphysalis* spp on goats based on light microscopic morphological features. However, the species spectrum of ectoparasites continually evolves with changes in time and climate (Prakasan and Ramani, 2007; Balasubramanian and Sahina, 2022; Sahina *et al*., 2024). In the realm of

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tick taxonomy and speciation, classifying closely related tick species often presents a complex challenge. This complexity is not limited to subspecies but also includes distinct species with remarkably similar morphological features. Such ambiguities are prevalent among various tick species, where differentiation based solely on visual attributes becomes challenging. Moustafa *et al*. (2024) emphasized the importance of integrating molecular, genetic, and morphological approaches to unravel the complexities of tick taxonomy, thereby enhancing our understanding of their ecological roles and disease transmission potential (Rathinam and Sidhik, 2022). The use of scanning electron microscopy (SEM) to investigate the fine structures of *Haemaphysalis* spp on goats in Kerala, which could aid in species distinction, has not been extensively performed.

In light of the increasing prevalence of theileriosis and anaplasmosis reported in small ruminants of south India (Nagaraj *et al*., 2019), this study aims to investigate the species spectrum of ixodid ticks infesting goats using gross morphological features and to document the ultrastructural morphology of the most prevalent tick species. This study serves as a platform for detailed tick surveillance on goats reared in households of Kerala, which is crucial for assessing the potential risk factors of fatal tick-borne pathogens in both animals and humans.

MATERIALS AND METHODS

Adult ticks (n=1200) were collected from 500 naturally infested goats in small and medium farms of south, central and north districts of Kerala and identified based on standard gross morphological keys (Sen and Fletcher, 1962; Trapido *et al.,* 1964). Adult goats and kids belonging to Malabari and Malabari cross breds were selected. For SEM studies, the ticks were dehydrated in series of ascending grades of ethyl alcohol (50 to 100%) and dried to critical point drying with CPD (Critical Drying Point) unit. The processed samples were mounted over the stubs with double sided carbon conductivity tape and a thin layer of gold coating was done by an automated sputter-coater for three minutes with vacuum conditions of pressure $1.5x10^{-3}$pa and accelerating voltage of 5-8 kV and scanned under Scanning Electron Microscope (SEM Model: TESCAN VEGA 3) at required magnifications as per the standard procedures at Central Instruments Laboratory, College of Veterinary and Animal Sciences, Mannuthy, Kerala. Ticks were scanned on the dorsal and ventral sides and the external features such as mouth parts, infra internal setae dentition, hypostome, adanal plates, festoons, scutum, porose area, shape of genital sucker, lateral groove and spiracles were observed.

RESULTS

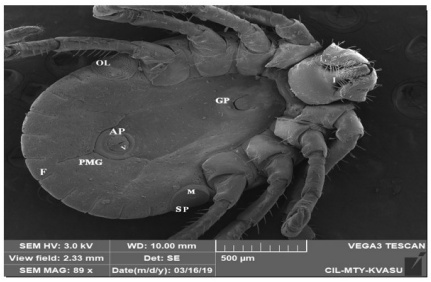
Out of the 1200 adult ticks examined, 1190 (99.1 %) belonged to *Haemaphysalis* genus and ten (0.83%) to *R. haemaphysaloides* based on gross morphological features. The characteristic morphological features of the prevalent tick genus, *Haemaphysalis* spp. under SEM were recorded. The most prevalent species on goats in Kerala as observed in the present study was *H. bispinosa* (n=747), followed by *H. intermedia* (n=435), *H. kutchensis* (n=4) and *H. megalaimae* (n=1). The species of three *Haemaphysalis* specimens could not be ascertained due to mutilation of mouth parts. (Table 1)

Table 1. Tick species distribution

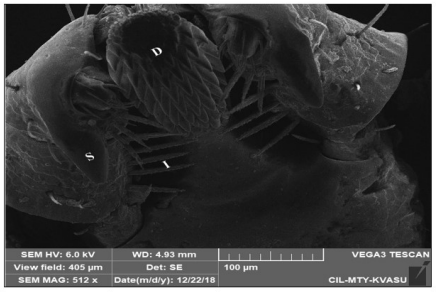
	Tick species	**Number (%) n=1200**
1	*Haemaphysalis bispinosa*	747 (62.25 %)
2	*H. intermedia*	435 (36.25 %)
3	*H. kutchensis*	4 (0.33%)
4	*H. megalaimae*	1(0.08 %)
5	*Rhipicephalus haemaphysaloides*	10 (0.83 %)

Mouth parts: Mouth parts comprising of a hypostome, paired chelicerae, two palps and the lateral prolongation of the second segment of pedipalp were distinctly seen in all the specimens of *Haemaphysalis* spp. as were evident in light microscopy itself. The chelicerae had two digits armed with sharp denticles. The hypostome had

1a) Hypostome



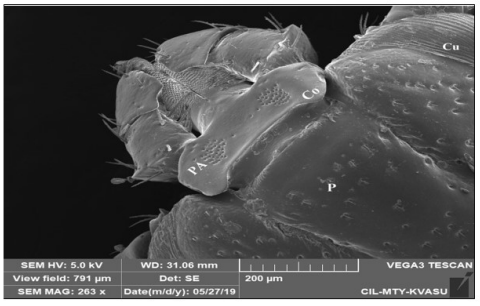
1b) *H. bispinosa*



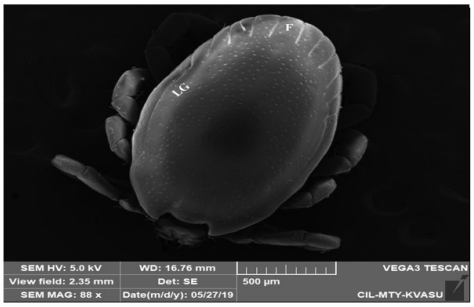
1c) *H. intermedia*

1d) *H. kutchensis*

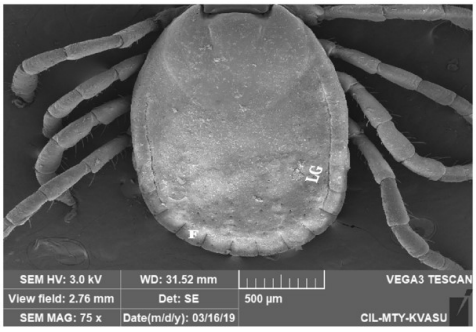
Fig. 1 Mouth parts of *Haemaphysalis* spp. a) Hypostome, b) *H. bispinosa,* c) *H. intermedia,* d) *H. kutchensis* AP- anal pore; D- dentition; F- festoon; GP- genital pore; M- macula; I- infrainternal setae; S- spur; SP-spiracle; OL- ostial lip; PMG- posterio-median groove



2a) Scutum



2b) Lateral groove of *H. intermedia*



2c) Lateral groove *H. bispinosa*

Fig. 2 Dorsal surface of *Haemaphysalis* spp. a) Scutum, b) Lateral groove *H. intermedia,* c) Lateral groove *H. bispinosa*: Co - cornua; Cu - cuticle; LG - lateral groove; PA - porose area; P - punctuations

recurved chitinous plates with minute denticles at the apex The dentition on the hypostome that play a key role in attachment to the hosts, was 4/4 in all the specimes (Fig. 1a).

The number and appearance of infra internal setae varied from 2 to 10 between species. *Haemaphysalis bispinosa* could be distinguished by the presence of four to five infra internal setae that were non-feathery and distinctly separate (Fig. 1b). The specific feature of the dorsal side of the palp of this species was the long and prominent dorsal retroverted spur (DS) on the palpal article 3 (P3) (Fig. 4). The lateral salience of palp 2 extended well beyond the lateral margin of palpal article 3. The third palpal segment revealed a ventral retroverted spur that was not reaching the basal margin of palp 2 (Fig.1b). Feathery and closely set six to seven infra internal setae could be distinctly associated with *H. intermedia* (Fig 1c-I) . Besides, in this species, the third segment of pedipalp revealed a ventral spur that was not reaching the basal margin of palp 2 (Fig. 1c-S). The dorsal spur of *H. intermedia* on palpal article 3 was short. Two specimens that possessed eight to ten feathery, closely set, infra internal setae were identified as *H. kutchensis*, while three could be observed in *H. megalaimae*. The rounded projection at the baso-internal angle of palpal segment 3 of *H. kutchensis* stretched beyond the internal margin of segment 2 The ventral spur on the third pedipalp of *H. kutchensis* was exceptionally long reaching the base of the palpal segment 2 (Fig. 1d- S).

Dorsal surface: Cuticle, at higher magnification, showed tiny surface folds giving a finger-print like appearance. On the dorsal surface of basis capitulum, porose areas were visible as a pair of circular depression containing several pores. The lateral cornua were moderately projected in all the specimens. The scutum had tiny pores or sensillaoriformia. The punctuations on the scutum of *H. kutchensis* were more piliferous when compared to other species (Fig. 2a). The lateral grooves enclosed the first festoon as was observed in *H. intermedia* (Fig. 2b), while it reached upto the second festoon in *H. bispinosa* (Fig. 2c).

Ventral surface: Genital aperture of females had a wide open U shape in all the studied species (Fig. 1b), except in *H. kutchensis*, in which it was narrow (Fig. 3a). The anal pore was made up of a central slit and two semicircular valves. A posterio-median groove extending between the anal groove and festoons was distinct in all specimens. In *H. kutchensis,* the posterio-median groove stretched into the level of festoons (Fig. 3a), while in *H. bispinosa* this groove terminated just above the level of festoons (Fig. 1b). The number of festoons varied from 10 to 12. The spiracles were broadly oval to circular in outline (Fig. 1b), the spiracular plate being elevated from the rest of the integument. There were more than 150 aeropyles of different sizes in each of the spiracle. A well distinct thick cuticular macula with a crescenteric ostial lip was discernible in the spiracles (Fig. 3b). The spiracles of *H. kutchensis* were elongate and oval in shape (Fig. 3b).

Haller's organ: The Haller's organ on the tarsus of ticks had a distinct anterior pit with several sensillae and a proximal capsule with a wavy slit. In the anterior pit one sensilla was longer than the rest. Several needle-like bristles were observed around the Haller's organ (Fig. 3c). The terminal portion of leg was armed with a globular adhesive pad like structure adjacent to a pair of curved claws. Ventral and dorsal plates of this pad could be observed. The pad reached almost upto the apical curvature of the claws (Fig. 3d).

DISCUSSION

The distribution of any vector borne pathogen in specific geographic regions is intricately linked to the distribution of its vectors in the particular area. *Haemaphysalis* spp. was the predominant species on goats in Kerala, a hot humid state of south India (Sahina *et al*., 2024). According to Moustafa *et al.* (2024), the taxonomy and genetic differentiation of *Haemaphysalis*, one of the most widespread genera in Asia, present considerable challenges. Accurate identification of tick species, often through morphological characteristics, is vital for public and veterinary health and for controlling associated tick-borne diseases. One of the key morphologic

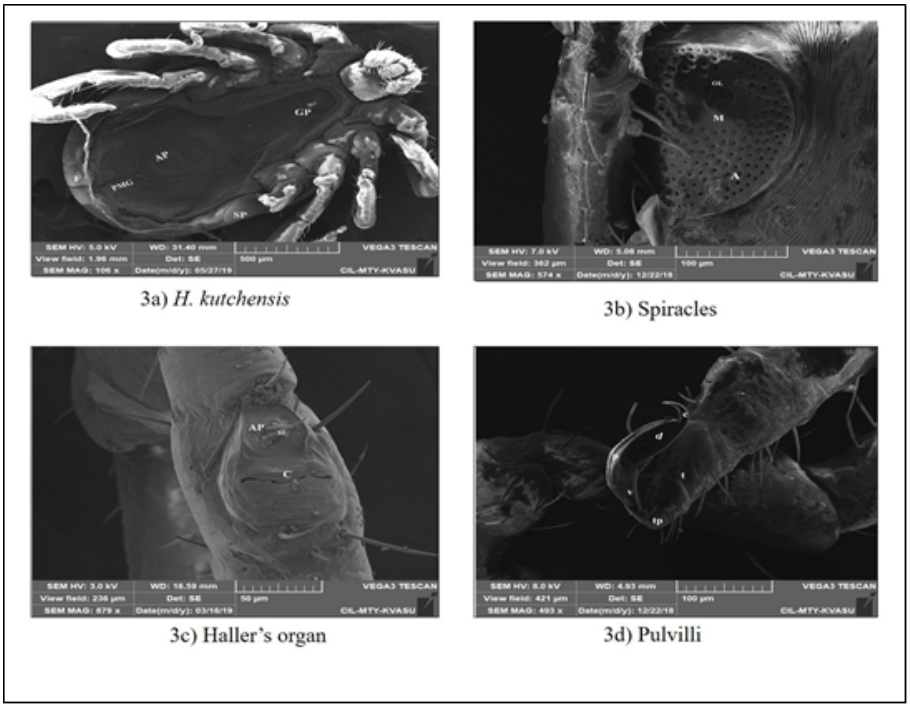


Fig. 3 Spiracles and Haller's organ of *Haemaphysalis* spp. a) *H. kutchensis,* b) Spiracles, c) Haller's organ, d) Pulvilli. A- aeropyle; C- capsule; c- claw; d –dorsal plate; SL- sensilla; t-tarsus; tp- tarsal-pretarsal articulation; v-ventro-lateral plate

Fig. 4 Dorsal view of *H.bispinosa* in light microscopy (Ds - Dorsal spur, P3 - Palpal segment 3)

characteristics used to differentiate ixodid ticks are the mouthparts (Thompson *et al.,* 2020). In this study, mouthparts were examined under scanning electron microscopy to identify the species. The dentition on the hypostome, which plays a crucial role in attachment to hosts, was 4/4 in all four species studied. Jin *et al.* (2016) had reported that male *H. tibetensis* possessed a 5/5 dentition pattern, while females had a 4/4 pattern. The number and appearance of infra internal setae varied from 2 to 10 between *Haemaphysalis* species, serving as a distinctive feature for different species within the genus. These observations concurred with the published keys (Trapido *et al.,* 1964). The length of the spurs on the third pedipalp also varied with species. The ventral spur on the third pedipalp of *H. kutchensis* was exceptionally long reaching the base of the palpal segment 2 as reported by Hoogstraal and Trapido (1963).

The punctuations on the scutum of *H. kutchensis* were found to be more piliferous when compared to other species. In *H. tibetensis*, the punctuations were minute and medium sized, as observed by Jin *et al.* (2016). According to Pegram *et al.* (1987) the punctuations on scutum of *R. sanguineus* were large, deep and piliferous being distributed in four uniform rows.

The lateral grooves enclosed the first festoon in *H. intermedia,* while it reached upto the second festoon in *H. bispinosa*. The reports on the lateral grooves reaching upto the level of coxa III in *H. kutchensis* and in *H. tibetensis* (Hoogstraal and Trapido 1963; Jin *et al.,* 2016) suggested that interspecies variations do exist in this feature as well.

Genital aperture of females had a wide open U shape in all the species studied, except in *H. kutchensis*. This is not a very distinguishing feature as the appearance of aperture in engorged and mated ticks differed from that in unmated ones (Hoogstraal and Trapido, 1963). The number of festoons in *Haemaphysalis* varied from 11 to 12. Caetano *et al.* (2017) had observed 11 festoons in *R. sanguineus*. The spiracles were broadly oval to circular in outline in all species of *Haemaphysalis*, while those of *H. kutchensis*, were more elongate. Woolley (1972) noted that the spiracular plates of *Ixodes* and *Haemaphysalis* were rounded in outline, while those of *Rhipicephalus* were subtriangular or comma shaped. There were more than 150 aeropyles of different sizes in each of the spiracles. Spiracular pores are reported to vary with different genera of ticks (Hinton, 1967; Roshdy and Hefnawy, 1973).

A well distinct Haller's organ with a proximal pit containing several sensillae and a capsule was observed on the tarsus in the first pair of appendages. Haller described this for the first time in 1881 as an auditory organ. Studies of Foelix and Axtell (1972) proved that anterior pit functioned as humidity receptor, while the capsule was involved in olfaction. The capsule of the Haller's organ of *Amblyomma* spp was not perforated and the number of slits varied with species (Ghosh *et al*., 2017). A narrow slit was reported in *A. americanum*, while *A. gervaisi* and *A. helvolum* had six and five slits, respectively. In the present study, the capsule of Haller's organ of *Haemaphysalis* spp. was found to have a single wavy slit.

The dorsal and ventral lobes of the tarsal pad were evident between paired tarsal claws. The claws of *H. tibetensis* were moderate in size and the pad did not extend upto the distal curvature of claws as reported by Jin *et al*. (2016), while it was reaching almost upto the apical curvature of the claws in all the *Haemaphysalis* species in the present study. The position of tarsal pad may, however, vary with the posture of fixing for the SEM study.

The present study puts on record the ultrastructural description of *Haemaphysalis s*pecies on goats in Kerala. Based on morphological features, *H. bispinosa* and *H. intermedia* were the most prevalent species on goats. The study area's local environment may favorable for the development of *H. bispinosa* (Sahina *et al*., 2024; Rathinam and Sidhik, 2022). Thus, goats are important reproductive hosts for these ixodid ticks in the study region. The study also recorded *H. kutchensis* and *H. megalaimae* for the first time in the state. The

role of these ticks as potential vectors of pathogens infecting animals and humans needs further investigation in the country. It is interesting to note that *H. spinigera*, the vector of Kyasannur Forest Disease in humans reported from Kerala (Babu *et al*., 2019), could not be observed on goats of this region. Genetic characterisation has been utilised to differentiate *H. cornupunctata*, *H. kashmirensis* and *H. montgomeryi* infesting goats and sheep in Pakistan (Khan *et al.,* 2023) The description of *Haemaphysalis* species, a potential vector of pathogenic infections would also serve as a platform to redesign the map of vector tick fauna on different species of livestock in this part of the country.

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Assessing the impact of foliar insecticides on yellow stem borer *Scirpophaga incertulas* and rice yield enhancement

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ABSTRACT: The field efficacy of various foliar insecticides with different modes of action was carried out against yellow stem borer during summer 2023 at CoA, V.C. Farm, Mandya. Among the treatments, flubendiamide 39.35 SC emerged as the most effective treatment, followed by chlorantraniliprole 18.5 SC and fipronil 5 SC with the least dead heart and white ears. Flubendiamide also recorded the highest grain and biomass yield followed by chlorantraniliprole 18.5 SC and fipronil 5 SC. Additionally, the economic analysis revealed that among the evaluated insecticide treatments, flubendiamide 39.35 SC demonstrated the highest net returns (Rs. 150819 per hectare) and benefit-cost ratio (4.44).
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KEY WORDS: Paddy, diamides, bio-efficacy, *dead hearts, white ears,* yield, cost-economics

INTRODUCTION

Rice, frequently referred to as the cornerstone of sustenance, holds immense significance worldwide, acting as the main source of nourishment for more than half of the global population (Rai, 2006; Pathak *et al*., 2018). The crop is predominantly cultivated in India, followed by China and Indonesia; however, China leads in overall production, while Australia boasts the highest productivity rates. In India, rice is grown over an area of 46.38 million hectares, producing 130.29 million tonnes and productivity of 2809 kg per hectare (Indiastat, 2022). In a country where rice is vital for food security, its cultivation covers extensive regions, with significant contributions from states such as West Bengal, Uttar Pradesh, Punjab, Telangana, Odisha, and Karnataka (Indiastat, 2022). Within Karnataka, rice cultivation occurs across 27 districts, with an area of 1.39 million hectares, production of 4.31 million tonnes and productivity of 3089 kg per hectare (MoA&FW, 2022). Nonetheless, several biotic and abiotic factors have been recognized as significant limitations that hinder the potential yield of rice, resulting in considerable decreases in quality and quantity.

The intensification of rice cultivation practices, marked by the widespread adoption of high-yielding varieties and monocropping, exacerbates pest incidences, exacerbating losses (Neeta *et al*., 2013). Among different groups of insect pests, stem borer (40%), planthopper (25%), gall midge (10%) and other pests (25%) are ranked in order of the indicated per cent share of the total losses caused by insect pests together (Reddy *et al*., 2010).

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Furthermore, pest outbreaks can lead to complete crop failure or severe yield loss. Amongst the stem borers that feed on rice, yellow stem borer (YSB) *Scirpophaga incertulas* (Walker) is the most destructive pest causing about a 25-30 per cent reduction in yield. This results in an annual yield loss of 27-34 per cent of the production (Pasalu *et al*., 2002; Chatterjee and Mondal, 2014). Attack by stem borers at the vegetative stage of plant growth produces symptoms called 'dead hearts' (DHs). In contrast, attack at the reproductive stage produces 'whit ears' (WEs). Rice plants can compensate for low incidences of DHs, but a 1–3 per cent loss of yield is expected for every per cent of WEs (Pathak *et al*., 1971; Balaji, 2024).

Insecticides provide an effective approach to controlling pests. Their application positively influences rice yields (Abro *et al*., 2013) and is characterized by being highly effective, fast-acting, convenient, and cost-effective, making them essential tools in pest management. It is crucial to utilize selective insecticides that target the YSB while being less harmful to beneficial species. However, excessive reliance on conventional insecticides has led to the development of resistance, highlighting the need to investigate new insecticide molecules (Pasalu *et al*., 2002; Singh and Rajshree, 2017). Developing and implementing novel insecticide molecules for managing the paddy yellow stem borer is vital for tackling resistance, enhancing efficacy, reducing environmental impact, promoting sustainability, and protecting rice production. In this regard, evaluating insecticide molecules is of utmost importance. By assessing their effectiveness against YSB infestation, their effects on yield attributes, and their cost-benefit ratios, this study aims to enhance the range of pest management strategies crucial for ensuring rice production and global food security.

MATERIALS AND METHODS

The field experiment was conducted during *Summer* 2023 at "A" block, College of Agriculture, V.C. Farm Mandya (12.5690° N, 76.8107° E) to evaluate new and conventional foliar insecticides. The trial consisted of nine treatments, including control (Table 1) with three replications and the treatments were imposed following Randomized Complete Block Design. The susceptible and popular rice variety Jaya was sown and 25 days old seedlings were transplanted in replicated blocks of 4 x 3 m with the spacing of 20 x 15 cm between rows and plants respectively. The crop was raised as per packages of practices except for the management schedule for YSB (POP, 2017). In each treatment, the recommended insecticides were applied based on ETL *i.e*., 5 per cent DH during the booting stage or 1 egg mass m^{-2} (Asghar *et al*., 2009) and the second spray was initiated at 90 DAT. In each spray, per cent DH and WEs were observed a day before application (to compare pre- and post-spray differences and calculate per cent infestation reduction) and 5, 10, 15 and 20 days after application on ten hills using the Standard Evaluation System for Rice (SES) formula (IRRI, 2013).

$$\% \text{ Dead heart} = \frac{\text{Total No. of dead heart per 10 hill}}{\text{Total No. of tillers}} \times 100$$

$$\% \text{ White ears} = \frac{\text{Total No. of white ears per 10 hills}}{\text{Total No. of panicles}} \times 100$$

Data on percent DH and WEs were analyzed in Microsoft Excel 2021, arc sin-transformed, and subjected to ANOVA (Gomez and Gomez, 1984; Hoshmand, 1988), with means separated by Tukey's HSD (Tukey, 1965). Results are graphically represented using GraphPad Prism 8.0.2 for better interpretation.

Grain yield and cost economics: Grain yield was recorded at physiological maturity and converted to quintals per hectare. Grain and biomass yields were analyzed using ANOVA (Gomez and Gomez, 1984; Hoshmand, 1988), with means separated by Tukey's HSD (Tukey, 1965). The percent additional yield gain over the untreated control was calculated for each treatment.

$$\text{Per cent additional grain yield} = \frac{(\text{Yield in treatment} - \text{Yield in control})}{\text{Yield in control}} \times 100$$

The cost economics of insecticidal treatments was analyzed using prevailing market prices of insecticides, labor wages, and additional costs, with the benefit-cost ratio calculated accordingly.

$$\text{Benefit Cost ratio} = \frac{\text{Net profit (Rs)}}{\text{Total cost (Rs)}}$$

RESULTS AND DISCUSSION

All the insecticides significantly reduced the dead heart infestation compared to the control at 5, 10, 15 and 20 days after treatment in the first application. The first application was done 35 days after transplanting and one day before treatment the mean dead heart ranged from 15.88 to 17.16 per cent during summer 2023 and there were no statistical differences among the treatments. In all the treatments at 20 DAS, the per cent dead heart was reduced (Table 1). Among all the treatments the minimum per cent dead heart was observed in flubendiamide 39.35 SC (4.18%), followed by chlorantraniliprole 18.5 SC (5.83%) which was on par with fipronil 5 SC (6.48%). The next best treatments were thiamethoxam 25 WG (7.22%), carbosulfan 25 EC (7.83%), quinalphos 25 EC (8.16%), cartap hydrochloride 50 SP (9.92%) and lambda cyhalothrin 2.5 EC (11.15%). Significantly higher per cent dead hearts was observed in the control (23.32%) (Table 2). At 90 DAT, the second spray was taken and per cent white ears were recorded. One day before spraying the mean white ears ranged from 12.56 to 15.43 per cent. There were significant statistical differences between the treatments and the control (Table 2). At 20 DAS, a substantial and lower per cent white ear was observed in flubendiamide 39.35 SC which recorded 4.88 per cent white ears followed by chlorantraniliprole 18.5 SC (5.41%) and fipronil 5 SC (5.81%). Significantly higher per cent white ears was observed in the control which recorded 20.12 per cent. Similar results were reported by Devi and Singh (2016), where they found flubendiamide 39.35 SC @ 24g a.i. ha^{-1} as the most effective molecule against *S. incertulas* in both 2013 and 2014, with the lowest percentages of dead heart (3.48 and 3.49) and white ear (1.32 and 1.33). Likewise, previous workers reported that lowest

Table 1. Bio-efficacy of foliar insecticides against *S. incertulas*, summer (I Spray)

No.	Treatments (dose @ ml or g l^{-1})	Dead hearts (%)				
		1 DBS	5 DAS	10 DAS	15 DAS	20 DAS
1	Cartap hydrochloride 50 SP (2)	16.65	12.21	12.00	10.30	9.92
		(24.09)^{a}	(20.46)^{bc}	(20.28)^{bc}	(18.73)^{bc}	(18.37)^{bc}
2	Carbosulfan 25 EC (1.5)	17.14	11.24	10.44	8.98	7.83
		(24.47)^{a}	(19.6)^{bcd}	(18.86)^{cde}	(17.45)^{bc}	(16.26)^{de}
3	Chlorantraniliprole 18.5 SC (0.3)	16.88	9.13	8.82	6.52	5.83
		(24.27)^{a}	(17.6)^{de}	(17.29)^{ef}	(14.8)^{de}	(13.98)^{f}
4	Fipronil 5 SC (2)	16.05	10.00	9.34	7.72	6.48
		(23.63)^{a}	(18.44)^{cd}	(17.81)^{def}	(16.14)^{cd}	(14.75)^{ef}
5	Lambda cyhalothrin 2.5 EC (1)	16.06	13.65	12.67	11.53	11.15
		(23.63)^{a}	(21.69)^{b}	(20.87)^{b}	(19.86)^{b}	(19.52)^{b}
6	Thiamethoxam 25 WG (0.3)	16.40	10.10	10.09	8.66	7.22
		(23.9)^{a}	(18.54)^{cd}	(18.53)^{cde}	(17.13)^{cd}	(15.59)^{def}
7	Quinalphos 25 EC (3)	16.01	11.60	11.08	9.95	8.16
		(23.6)^{a}	(19.92)^{bc}	(19.46)^{bcd}	(18.39)^{bc}	(16.61)^{cd}
8	Flubendiamide 39.35 SC (0.15)	(15.88	7.85	7.87	5.29	4.18
		23.49)^{a}	(16.28)^{e}	(16.3^{f}	(13.31)^{e}	(11.81)^{g}
9	Untreated control	17.16	19.28	20.23	21.76	23.32
		(24.49)^{a}	(26.06)^{a}	(26.74)^{a}	(27.82)^{a}	(28.89)^{a}
SE m ±			0.42	0.37	0.52	0.36
CD (p = 0.05)		NS	1.26	1.10	1.57	1.07

DBS-Day before spray; DAS- Days after spray; Figures in the parenthesis indicate arc sin transformed values; Values in the column followed by common letters are non-significant at p=0.05 as per Tukey's HSD (Tukey, 1965)

Table 2. Bio-efficacy of foliar insecticides against *S. incertulas*, summer (II Spray)

No.	Treatments	White ears (%)				
		1 DBS	5 DAS	10 DAS	15 DAS	20 DAS
1	Cartap hydrochloride 50 SP	12.56	10.35	9.37	10.30	8.47
		(20.77)^{b}	(18.78)^{bc}	(17.83)^{bc}	(16.97)^{bc}	(16.93)^{b}
2	Carbosulfan 25 EC	13.28	8.95	8.57	8.98	6.96
		(21.38)^{b}	(17.42)^{cde}	(17.03)^{bcd}	(15.86)^{cde}	(15.31)^{c}
3	Chlorantraniliprole 18.5 SC	13.61	7.39	6.66	6.52	5.41
		(21.66)^{b}	(15.78)^{ef}	(14.97)^{e}	(14.1)^{ef}	(13.45)^{ef}
4	Fipronil 5 SC	13.27	7.81	7.24	7.72	5.81
		(21.37)^{b}	(16.24)^{ef}	(15.62)^{de}	(14.88)^{def}	(13.96)^{de}
5	Lambda cyhalothrin 2.5 EC	13.01	11.26	10.59	11.53	9.26
		(21.15)^{b}	(19.61)^{b}	(19)^{b}	(18.13)^{b}	(17.73)^{b}
6	Thiamethoxam 25 WG	13.52	8.47	7.86	8.66	6.68
		(21.59)^{b}	(16.93)^{def}	(16.28)^{cde}	(15.37)^{cde}	(14.99)^{cd}
7	Quinalphos 25 EC	12.91	9.90	8.82	9.95	7.12
		(21.07)^{b}	(18.35)^{bcd}	(17.28)^{bcd}	(16.15)^{cd}	(15.49)^{c}
8	Flubendiamide 39.35 SC	13.08	6.97	6.14	5.29	4.88
		(21.21)^{b}	(15.32)^{f}	(14.35)^{e}	(13.33)^{f}	(12.77)^{f}
9	Untreated control	15.43	17.57	18.32	21.76	20.12
		(23.14)^{a}	(24.79)^{a}	(25.36)^{a}	(25.53)^{a}	(26.67)^{a}
	SE m ±	0.28	0.33	0.41	0.39	0.23
	CD (p = 0.05)	0.85	0.98	1.21	1.16	0.69

DBS-Day before spray; DAS- Days after spray; Figures in the parenthesis indicate arc sin transformed values; Values in the column followed by common letters are non-significant at p=0.05 as per Tukey's HSD (Tukey, 1965)

incidence of YSB was seen in the chlorantraniliprole 18.5 SC (1.75%) treated plot, followed by fipronil 5 SC (2.59%) (Kinjale *et al*., 2021). Fipronil 5 SC reduced both dead heart and white ears after the application, at 3, 10 and 15 days, respectively (Abhinandan and Gupta, 2020). Similarly, Singh and Rajshree (2017) found flubendiamide 39.35 SC @ 24 g a.i./ha as effective, followed by chlorantraniliprole 18.35SC @ 30 g a.i./ha and thiamethoxam 25 WG @ 25 g a.i./ha. The per cent reduction ranged from 71.36 to 45.40 per cent. A Similar trend was found in the case of white ear infestation (Fig. 1).

Yield economics

The efficacy studies of insecticides against yellow stem borer showed significant variations in both grain and biomass yield. Flubendiamide 39.35 SC exhibited the highest grain yield (6.07 t ha^{-1}) and also the highest biomass yield (6.19 t ha^{-1}) followed by chlorantraniliprole 18.5 SC which recorded the next highest grain (5.88 t ha^{-1}) and biomass (6.06 t ha^{-1}) yield. The economic analysis on the feasibility of various treatments indicated that higher net returns of Rs. 150819 per hectare were achieved in flubendiamide 39.35 SC with the highest BC ratio of 4.44 (Table 3). The control showed a reduction of 2.24 t ha^{-1} when compared with the best insecticide treatment (flubendiamide 39.35 SC).

Recent advancements in the pesticide industry focussed on developing target-specific molecules like diamides, neonicotinoids, phenylpyrazoles and others. These new insecticides offer relatively low risk to non-target organisms and the environment and high target specificity, making them essential for environmentally safe integrated pest management and insect resistance management programs (Bhuvaneswari *et al*., 2022). The diamides are the most recent addition to the limited number of insecticide classes with specific target

Table 4. Cost economics of insecticides influenced by yellow stem borer in rice, summer 2023

No	Treatments	Yield (t/ha)		Total cost of cultivation (Rs/ha)	Gross return (Rs/ha)	Net profit (Rs/ha)	Yield advantage over control (t/ha)	B: C ratio
		Grain	Biomass					
1	Cartap hydrochloride 50 SP	4.68	4.78	34250	142531	108281	0.85	3.16
2	Carbosulfan 25 EC	5.14	5.37	34758	157766	123008	1.32	3.54
3	Chlorantraniliprole 18.5 SC	5.88	6.06	35800	179704	143904	2.05	4.02
4	Fipronil 5 SC	5.70	5.81	35260	173598	138338	1.87	3.92
5	Lambda Cyhalothrin 2.5EC	4.39	4.41	32846	133165	100319	0.56	3.05
6	Thiamethoxam 25 WG	5.29	5.35	32959	160741	127782	1.46	3.88
7	Quinalphos 25 EC	4.78	5.00	33850	146687	112837	0.95	3.33
8	Flubendiamide 39.35 SC	6.07	6.19	34000	184819	150819	2.24	4.44
9	Untreated control	3.83	3.90	32500	116611	84111		-

The price of paddy per quintal = Rs. 2180, price of fodder per quintal = Rs. 850

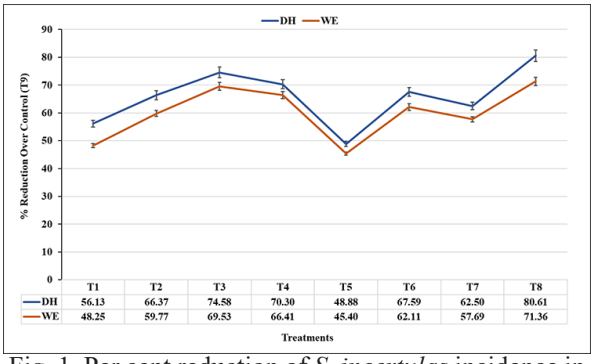

Fig. 1 Per cent reduction of *S. incertulas* incidence in different insecticidal treatments during summer. (DH – Dead heart; WE – White ears)

site activity that is highly efficacious, control a wide pest spectrum and have a favourable toxicological profile (Teixeira and Andaloro, 2013). Among diamides, flubendiamide and chlorantraniliprole have played a major role in preventing or delaying the evolution of insect resistance since the introduction of these novel insecticides. Flubendiamide is a phthalic acid diamide insecticide reported to target insects' ryanodine receptors (Richardson *et al*., 2020). In contrast to other insecticide classes targeting the insect nervous system, it acts at receptors in insect muscles causing an immediate cessation of feeding and thus avoiding crop damage (Ebbinghaus-Kintscher, 2007). Chlorantraniliprole binds to a specific receptor in muscles called the ryanodine receptor, which causes muscle cells to leak calcium leads to stop working the muscles normally; hence the insect would be paralyzed and die (NPIC, 2022).

The results of this study highlight the crucial role the novel insecticides, particularly flubendiamide 39.35 SC, chlorantraniliprole 18.5 SC and fipronil 5 SC, play in effectively managing the yellow stem borer in paddy cultivation. These insecticides not only significantly decreased the occurrence of dead hearts and white ears but also resulted in notable enhancements in grain and biomass yields. Flubendiamide demonstrated superior performance, yielding the highest grain output and net returns, indicating its potential as a leading solution for YSB management. Furthermore, the economic analysis underscores the effectiveness of these treatments in boosting both productivity and profitability for farmers.

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Foraging interaction of stingless bee *Tetragonula travancorica* with flowers in ornamental plants

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ABSTRACT: The foraging activity of the stingless bee (*Tetragonula travancorica*) was studied by observing flowers of 20 identified ornamental plants at their full bloom stage. The foraging preference of *T. travancorica* is primarily influenced by colour, size, shape, scent and the nutrient quality and availability of nectar and pollen. The Asteraceae family was the predominant group among the ornamental plants foraged by *T. travancorica*, followed by Rubiaceae, Malvaceae and Euphorbiaceae. Among the different types of hibiscus, *Malvaviscus arboreus* exhibited the highest foraging rate, potentially due to its high nectar availability (8.47 µl). The highest *T. travancorica* population was observed on red-colored ixora among different ixora varieties, likely due to its high nectar volume (1.73 µl) and sugar concentration. This study also indicated that *T. travancorica* prefers flowers with pollen containing high levels of protein and lipid. Among the foraged plants (*Antigonon leptopus, Malvaviscus arboreus, Rosa* spp., *Rosa bourboniana* and *Syagrus romanzoffiana*), *R. bourboniana* showed high protein and lipid content (7.613 and 324 mg ml^{-1}, respectively). © 2025 Association for Advancement of Entomology

KEY WORDS: Foraging resource, nectariferous, polleniferous, foraging preference

INTRODUCTION

Stingless bees, the smallest honey-producing bees, are highly social insects forming permanent colonies in concealed locations like old walls, logs, and crevices. Although their sting is significantly reduced and ineffective for defense, they can still bite to protect their hives (Aleixo *et al*., 2013). As eusocial bees native to the tropics and subtropics (Toledo-Hernandez *et al*., 2022), they play a crucial role in pollinating numerous tropical plant species, potentially contributing to about one-fifth of the local angiosperm flora (Wilms *et al*., 1996). In peninsular India, *Tetragonula travancorica* Shanas and Faseeh is a common species (Shanas and Faseeh, 2019). Despite their low honey yield per hive, its unique flavour and medicinal value drive high market demand and prices. Their abundance and diversity are vital for crop pollination in sustainable agriculture, ensuring food security.

Stingless bees are typically generalist foragers,

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efficiently collecting pollen and nectar from a wide variety of plants. Their visits to numerous species and families highlight their significant contribution to pollination (Aleixo *et al*., 2013). Their forage includes diverse sources such as fruits, vegetables, ornamentals, medicinal and forest plants, as well as weeds (Dalio, 2013). Across different regions, stingless bees readily utilize both exotic species within their preferred plant families (including crops, ornamentals, and weeds) and native plants (Bueno *et al*., 2023). Bee flora can be categorized into three types: nectar-only, pollen-only, and those providing both resources. Notably, stingless bees can exploit small, medium, and large tubular and non-tubular flowers for forage, demonstrating their ability to utilize flowers with varying qualities and quantities of floral rewards (Vamshikrishna *et al*., 2021). For most bee species, nectar serves as the primary carbohydrate source, containing sucrose, fructose, and glucose in varying ratios, along with trace amounts of essential oils, minerals, and other substances.

Pollen serves as the primary source of protein and essential amino acids for most bee species. It is crucial for their intake of proteins, lipids, and various micronutrients, including vitamins and minerals (Mokkapati *et al*., 2024). Bees rely on pollen for their physical development, producing food for the queen, nurturing larvae into adults, and maintaining the overall health and hygiene of their colony (Thakur and Nanda, 2020). Consequently, plants that offer both nectar and pollen are vital for beekeeping, providing the majority of the necessary nutrients through these floral resources. Foraging bees utilize visual and olfactory cues, along with learned preferences, to locate and select food sources (Dyer *et al*., 2019). Their choice of food is influenced by several factors, including scent marks, flower color, and location. Azuma *et al.* (2002) highlighted flower scent and its chemical profile as significant factors in attracting pollinators.

Hence to understand the inclination of *T. travancorica* in selecting food sources, a study was conducted considering factors like colour, shape, smell, nectar content, nectar sugar concentration, and pollen availability.

MATERIALS AND METHODS

Study site: The present work was conducted during September 2021 to August 2022 at College of Agriculture Vellayani, Kerala, India which lies within coordinates of 8.47^0 N and 76.98^0 E having gardens with native and exotic plants used in urban landscaping. Stingless bee foraging activity was studied weekly throughout the year by placing one hive near the field with different ornamental plants during the flowering period.

Identification of floral resources and characteristics: To identify the major flora visited by stingless bee, the flora encountered by the bees either for pollen or nectar or for both were observed regularly in and around the campus and documented. Pollen collectors were identified by the pellets of pollen adhering to their hind legs. Nectar collectors were identified by observing their abdomen, it is filled with nectar or those without pollen load in their hind leg were considered as nectar foragers. For determining flower color, ten fresh flowers were selected randomly. Flower color was identified using the color chart of Royal Horticultural Society. Flower shape was identified by selecting ten flowers randomly as described in www.theseedsite.co.uk.

Foraging preference of *Tetragonula travancorica*: The flowering plants near the hive were observed and the observations on foraging preference were recorded based on their activities. Ten flowers were observed for 10 minutes at weekly intervals from the identified plants at full bloom stage and their flowering period and floral characteristics were recorded. Nectar volume and sugar concentration was also assessed.

Pollen samples were analyzed for protein, lipid, phenol and flavonoid. Chemical composition of pollen were determined. Pollen was collected from identified plants randomly. Composite sampling was done from different flowers of same plant. Two gram of dried pollen of each sample was taken and 15 mL of 70 per cent ethanol solution was added and then it was agitated for 30 min. After agitation, the solution was shaken for two min. Then it was centrifuged for 20 min at 3500 rpm, at 28°C. The

supernatant was separated and stored at 4°C.

Total protein present in sample was estimated as per the procedure given by Lowry *et al*. (1951) and lipid by Evans and Needham (1987). Total phenol and flavonoid was estimated the procedure given by Singleton and Rossi (1965) and Ordonez *et al.*(2006).

Chemical composition of pollen samples was determined by using Gas-chromatograph coupled with mass spectrometer (GC-HRMS), model AccuTOF GCV and specification of model is electron impact EI / CI with Mass range of 10 - 2000 amu and 6000amu mass resolution. For GC, quartz capillary column of dimensions 30mm × 0.25mm × 0.25mm was used. The column temperature and flow rate were 60°C and 1.0 mL/ min respectively. The inlet temperature and heating rate were 250 °C and 6°C /min respectively. Pre column pressure and split ratio were 80 KPa and 40:1 respectively. The helium gas was used as carrier gas. For determining the composition of sample, the oven was set to 280°C, but the actual temperature was only 250°C.

One-way ANOVA was used to analyze the data about foraging activity which was carried out by using statistical software, GRAPES Version1.1.0.

RESULTS AND DISCUSSION

The present study documented *T. travancorica* foraging on 20 ornamental plant species (Table 1). Of these, eight species exhibited year-round flowering (*Antigonon leptopus, Hamelia patens, Portulaca grandiflora, Nymphaea* sp., *Malvaviscus arboreus, Caesalpinia pulcherrima, Combretum constrictum,* and *Jatropha pandurifolia*), while the remaining twelve were seasonal bloomers (*Rosa bourboniana, Rosa* spp., *Syagrus romanzoffiana, Pereskia aculeata, Helianthus annuus, Zinnia elegans, Tecoma capensis, Ixora chinensis, Tagetes erecta* and *Euphorbia milii*).

The interactions between foraging bees, specifically *T. travancorica*, and flowers are influenced by a combination of factors, including color, shape, smell, nectar content, sugar concentration, and pollen availability. *T. travancorica* uses tactile, olfactory, and visual cues to select suitable host plants for foraging. Floral parameters such as size, shape, colour, and pattern significantly impact pollinator attraction, as well as their landing and orientation behaviour. This experiment revealed that flowers with strong orange (15%), yellowish-white (15%), strong red, and vivid reddish-orange (14%) hues were most prevalent among the bee flora. These bees showed a preference for cup, funnel, and tubular flower shapes (20%) that offered easily accessible nectar and pollen. Other attractive shapes included bowl, kidney, and globose. In this study, the majority of plant species foraged by *T. travancorica* for nectar and pollen belonged to the Asteraceae family (21%), followed by Rubiaceae (11%), Euphorbiaceae (11%), and Malvaceae

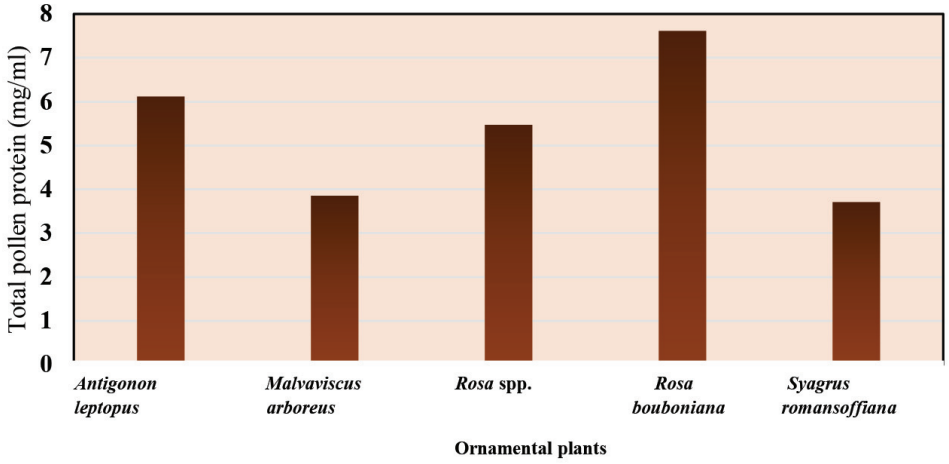

Fig. 1 Total protein content of pollen in different ornamental plants

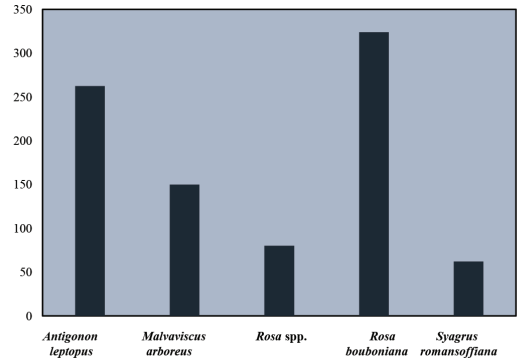

Fig. 2 Total lipid content of pollen in different ornamental plants

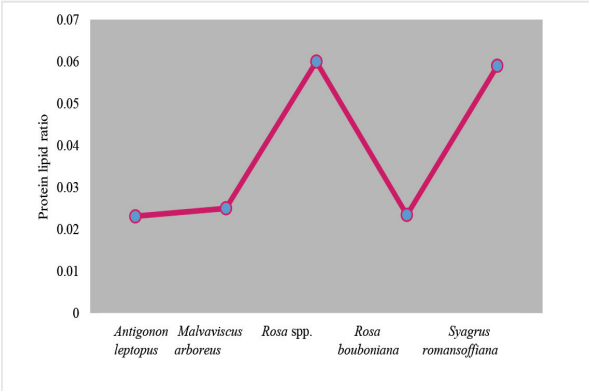

Fig. 3 Protein-lipid ratio of pollen in different ornamental plants

(11%). This aligns with Martins *et al*. (2023) findings, which identified Myrtaceae, Asteraceae, Euphorbiaceae, Melastomataceae and Malpighiaceae as dominant plant families preferred by stingless bees.

Antigonon leptopus was notable among the observed plants for its year-round nectar production, vigorous growth, and abundant flowering with a high nectar refill rate. The numerous bunched flowers offered an attractive resource, facilitating frequent nectar foraging for bees. While pollen was also available, stingless bees primarily favoured the nectar, consistent with Raju *et al.* (2001) observation of diverse insect visitors, including bees, wasps, flies, and butterflies, to these flowers. *Hamelia patens*, another year-round bloomer, attracted bees from afar with its showy, conspicuous, and bright aggregated flowers. The close arrangement of multiple flowers allowed efficient nectar collection in a single visit. Even with its long tubular corolla and concealed nectar, short-tongued bees could access the resource via capillary action. Likewise, the mass flowering of *Caesalpinia pulcherrima* and *Combretum constrictum* provided a reliable caloric reward for stingless bees, reducing foraging time, a preference also noted by Ramalho (2004) for mass-flowering plants. This aligns with Akter *et al*. (2017) finding that plants with numerous flowers and large clusters are generally more attractive to flower-visiting insects.

The experiment indicated a strong attraction of stingless bees towards fragrant flowers. Although color is a known visual attractant, the foraging rates observed on different colored roses showed that *R. bourboniana* (1.775) attracted the highest number of bees, suggesting fragrance as a key factor (Table 2). This supports Van *et al*. (2023) finding that plants employ varied floral displays in size, shape, color or fragrance to attract pollinators. Floral scent, as reported by Dudareva and Pichersky (2006), is a complex blend of volatile compounds produced by different floral tissues. Furthermore, Azuma *et al*. (2002) identified flower scent and its chemical profiles as significant attractants for pollinators.

Nymphaea sp. and *Portulaca grandiflora* are pollen-providing plants that flower throughout the year. *Nymphaea* offers easily accessible pollen due to its exposed floral structure. However, its stigmatic watery fluid, despite lacking sugar, poses a hazard as bees were seen to inadvertently fall and become trapped. This observation echoes

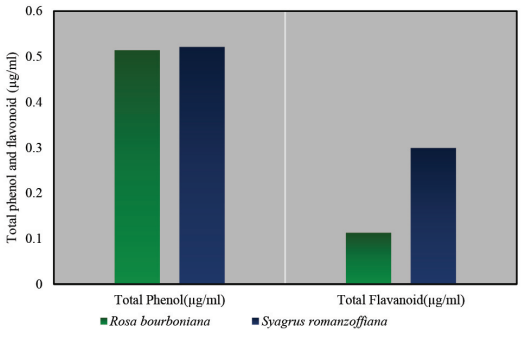

Fig. 4 Total phenol and flavonoid content of pollen in different ornamental plants

Table 1. Flowering season and flower characteristics of nectariferous and polleniferous ornamental plants

Common name	Scientific name	Family	Colour of the flower	Shape of flower
Coral vine	*Antigonon leptopus* Hook. and Arn.	Polygonaceae	Moderate purplish pink	Kidney shaped
Scarlet bush	*Hamelia patens* Jacq	Rubiaceae	Vivid reddish orange	Tubular
Moss rose/sun plant	*Portulaca grandiflora* L.	Portulacaceae	Light yellow, purplish pink	Saucer
Water lilly	*Nymphaea* sp.	Nymphaeaceae	White/Violet/Light pink	Cup like flower
Rose	*Rosa* spp.	Rosaceae	Greenish white	Cup like bloom
Scented Rose	*Rosa bourboniana* L.	Rosaceae	Light purplish pink	Cup like bloom
Ornamental palm	*Syagrus romanzoffiana* (Cham.)	Palmae	Yellowish white	Star shaped
Lemon vine	*Pereskia aculeata* L.	Cactaceae	Yellowish white	Cup like flower
Marigold	*Tagetes erecta* L.	Asteraceae	Strong orange	Head
Sunflower	*Helianthus annuus* L.	Asteraceae	Brilliant yellow	Capitulum
Hibiscus	*Malvaviscus arboreus* B.	Malvaceae	Strong red	Tubular
Hibiscus	*Hibiscus rosa-sinensis* L.	Malvaceae	Strong purplish red	Trumpet
Peacock plant	*Caesalpinia pulcherrima* L.	Fabaceae	Vivid reddish orange	Butter fly shaped
Chinese powder puff	*Combretum constrictum* B.	Combretaceae	Strong reddish orange	Globose
Chinese ixora (Red)	*Ixora chinensis* L.	Rubiaceae	reddish orange	Tubular
Ixora(Yellow)	*Ixora chinensis* L.	Rubiaceae	Pale yellow	Tubular
Cape honey suckle	*Tecoma capensis* L.	Begoniaceae	Strong orange	Funnel shaped
Peregrina	*Jatropha pandurifolia* L.	Euphorbiaceae	Strong red	Funnel shaped
Euphorbia	*Euphorbia mili* L.	Euphorbiaceae	white, Moderate red	Bowl shaped

Robertson's (1889) findings on *N. odorata*, where a tasteless and odorless fluid in the stigmatic bowl led to accidental insect falls. This contrasts with Zhang *et al*. (2021) study on the bright *Nymphaea* hybrid, which used an aromatic odour for attraction and secreted colourless, tasteless nectar on the stigma.

Portulaca grandiflora provides a consistent year-round pollen source, with stingless bees preferring the easily accessible single-petaled flowers over the pollen-concealing multi-petaled types. Seasonally, *Syagrus romanzoffiana* offers abundant pollen from its numerous yellowish-white flowers on branched inflorescences (45-50 rachis with 78-100 flowers each). This large pollen availability reduces bee search time and energy, leading to frequent and rapid visits. The efficiency of stingless bees as pollinators of *Syagrus* species is highlighted by Nunez Avellaneda and Carreno (2017), who found them to transfer an average of 83 per cent of the pollen in *S. orinocensis*.

The findings indicate that stingless bee foraging is a complex process influenced by a combination of floral characteristics, including color, size, shape, scent, and importantly, the nutrient quality and quantity of nectar and pollen. Contrary to the general preference for cup-like flowers, this experiment showed that tubular flowers with high nectar volume are also attractive. For instance, among the studied hibiscus species (Table 2), the tubular *M. arboreus* exhibited the highest foraging rate (4.41), likely due to its high nectar volume (8.47 µL) and sugar content (23.06%), providing a substantial energetic reward. *Ixora chinensis*

Table 2. Foraging preference of *Tetragonula travancorica* on different coloured roses, hibiscus and ixora (Mean numbers visited per 10 minute)

Rose	Number
Scented rose (*R. bourboniana*)	1.775[a]
White rose (*Rosa* spp.)	1.719[ab]
Red rose	1.032[bc]
Orange rose	1.104[c]
Pink rose	1.242[c]
Yellow rose	0.958[c]
CD(0.05)	0.505
Hibiscus	
Malvaviscus arboreus	4.47[a]
Hibiscus rosasinensis	2.529[b]
CD(0.05)	0.663
Ixora	
Red Ixora	2.934[a]
Yellow ixora	2.023[b]
CD(0.05)	0.231

served as a source of both pollen and nectar for these bees. Furthermore, among the ixora varieties (Table 2), red and yellow Chinese ixora attracted the most bees, with the red variety hosting the highest population (2.934), a trend strongly correlated with its nectar volume (1.73 µL) and sugar concentration (16.73%) (Table 3).

Protein and lipids obtained from pollen are essential for the growth and development of bee larvae, as well as the health and reproduction of adult bees. Ghosh *et al.* (2020) found that honey bees prioritize pollen based on its protein content. Our study similarly reveals that stingless bees prefer flowers with pollen that is high in both protein and lipids, and also has a high protein-to-lipid ratio. *R. bourboniana*, the most frequently foraged plant, had high protein and lipid levels (7.613 and 324 mg/mL) (Fig. 1, 2), while *S. romanzoffiana*, a plant heavily visited for pollen, displayed a high protein-to-lipid ratio (Fig. 3). The protein-to-lipid ratios in pollen seem to be a key factor in stingless bee foraging preferences among pollen-providing plants, and these preferred ratios are associated with the health and vitality of bee colonies (Vaudo *et al*., 2020). Preferred *R. bourboniana* (0.514 and 0.113 µL) and *S. romanzoffiana* (0.521 and 0.299 µL), also had high phenol and flavonoid content compared to other pollen sources (Fig. 4). This aligns with Campos *et al*. (1997) finding that red and blue anthocyanin pigments (flavonoids) attract insect pollinators, and Liu *et al*. (2006) report that changes in pollen phenolic content influence honey bee foraging activity.

The chemical composition of pollen appears to be a significant factor influencing the foraging behavior of stingless bees, as observed in this study. This composition likely determines the amount of pollen gathered and the probability of a bee revisiting similar flowers. Our analysis of pollen from frequently foraged plants identified several chemical components with potential attractive properties: Hexadecanoic acid ethyl ester, Docosanoic acid, 1, 2, 3-propanetriylester, and Benzene dicarboxylic acid, butyl 1, 2-ethylexylester, methyl ester. This finding supports the assertion by Dobson and Bergstrom (2000) that chemicals in pollen play a crucial role in how bees recognize their host plants.

Our study contributes to the growing understanding that bee foraging interactions with flowers are a

Table 3. Mean nectar volume and sugar concentration of major nectar yielding ornamental plants

Ornamental plants	Nectar (µl)	Sugar (%)
Antigonon leptopus	Trace	8.47[e]
Hamelia patens	1.50[c]	25.45[b]
Malvaviscus arboreus	8.47[a]	23.06[c]
Ixora chinensis	1.73[b]	16.73[e]
Caesalpinia pulcherrima	0.66[e]	24.00[a]
Combretum constrictum	1.00[d]	17.73[d]
CD (0.05%)	0.018	0.32

complex process influenced not only by visual and olfactory cues, as well as individual bee preferences, but also critically by the nutrient quality and availability of nectar and pollen. Additionally, observed that the aggregated structure of flowers plays a crucial role in attracting a large number of bees from afar, enabling them to efficiently collect sufficient nectar during each visit, thus minimizing energy expenditure.

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In Silico biological evaluation of propolis for modulating Ghrelin, Leptin and CYP17A1 gene pathways in Polycystic Ovary Syndrome

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ABSTRACT: The study focused on identifying the potential phytochemicals from the propolis, a beehive byproduct, as a modulator in Ghrelin, Leptin and CYP17A1 gene pathways. From ADMET and molecular docking analysis, Quercetin and Chrysin show good binding interactions with target receptors and obey drug-like properties compared to the standard drug Lorcaserin. The Molecular dynamics simulation of protein-ligand complexes revealed Quercetin-binding proteins are slightly lower RMSD indicating a stable association of Quercetin with the Ghrelin receptor. © 2025 Association for Advancement of Entomology

KEY WORDS: Docking, molecular dynamics, toxicity, molecules screening analysis

INTRODUCTION

Propolis is a beehive byproduct that has been used as a natural remedy for centuries. It has antimicrobial, anti-inflammatory and antioxidant properties and has many health benefits. Propolis is widely used to treat ulcers and swelling in the mouth, cold sores, and diabetes. Additionally, it is used to treat wound healing, genital herpes, burns, and canker sores. (Ashmitha *et al.,* 2024). Aside from these potentially beneficial substances, propolis also includes minerals like magnesium and potassium, as well as amino acids, vitamins A, C and E (Cirasino *et al.,* 1987). Specifically, propolis contains polyphenols called flavonoids. In addition to its flavonoid concentration, it possesses anti-allergy, immunomodulatory, anti-cancer, anti-fungal, and anti-ageing properties (Castaldo *et al.,* 2002). Propolis has been demonstrated in studies to control ovarian hormone function as well as promote digestive system development and function. Polyphenols are antioxidants that fight disease and damage in the body (Sapmaz *et al.,* 2022). In this study, we aimed to identify the potential bioactive molecules in propolis to target the Ghrelin, Leptin, and CYP17A1 gene receptors using ADMET, molecular docking, and molecular dynamics simulation approaches. It's a new avenue way to control PCOS issues.

MATERIALS AND METHODS

ADMET analysis: The structure of compounds present in propolis was obtained from PubChem and While conducting docking research, it is crucial to assess the drug-likeness property using the Qik

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prop module to forecast their draggability property, such as the Absorption, Distribution, Metabolism Excretion, and Toxicity (ADMET). The CNS, blood-barrier coefficient, human blood absorption, and Lipinski's rules of three and five were among the significant characteristics that were examined. The bioactive chemicals that demonstrated practical outcomes were selected for the ADMET, and the docking rationale has been tabulated with the preferred docking poses (Puratchikody *et al.,* 2019).

Molecular docking: The 3D structure of obesity-responsible proteins was retrieved from the Protein Data Bank (www.rcsb.org). The protein structure was initially created using the BIOVIA DS visualiser's Prepare protein setup before the docking tests. Preparing the protein is an optimisation procedure that addresses charge anomalies, atomic and bond lengths, and structure. The protein structure was cleared of water molecules and the first inhibitor. Using Maestro v10.2's protein residue template, hydrogens were inserted again. After that, the optimisation phase was used to produce stable conformation and docking analysis was performed by converting to PDBQT format. The bio constituents Quercetin, Chrysin, Quercetin pentamethyl ether, 2, 4,4-Trimethyl, Ethyl alpha-d-glucopyranoside, Hexacosane, Capric acid, Carprofen, Tetracontane identified from Propolis were retrieved from PubChem chemical databases. The ligands were imported to the workspace Schrodinger software and preparation was done.

Molecular dynamics: M D simulations were performed using the Desmond package on the OPLS3e force field. As mentioned in other works, the simulation process and additional experimental data were used precisely (Balasubramaniyan *et al*., 2018). At 100 ps intervals during the 100 ns runtime of the experiment, trajectory snapshots were taken. M D trajectories were examined using the Desmond Simulation Interaction Diagram (SID) to predict the binding configuration and stability of the ligand.

RESULTS AND DISCUSSION

ADMET analysis: Each component of ADMET addresses a fundamental aspect of a drug's absorption through the human body. Absorption determines how effectively a drug enters the bloodstream from its site of administration. Distribution examines the extent and manner in which the drug spreads to various tissues and organs. Metabolism involves the biochemical modifications the drug undergoes, primarily in the liver, influencing its activity and duration of effect. Excretion covers the pathways and efficiency of drug elimination from the body, while toxicity assesses the potential adverse effects, ensuring safety at therapeutic doses. Optimizing these parameters helps in predicting the in vivo behavior of drug candidates, thereby reducing the risk of late-stage failures in clinical trials.

In parallel, drug-like properties are essential for ensuring that a compound not only exhibits the desired biological activity but also possesses favorable characteristics for development into a viable drug. Key properties consist of polar surface area, hydrogen bonding ability, lipophilicity, and molecule size which influence a drug's solubility, permeability, and overall pharmacokinetic profile. A balance between these properties is crucial; for instance, a compound needs to be lipophilic enough to cross cell membranes yet not so lipophilic that it becomes insoluble in aqueous environments. Similarly, appropriate molecular size and limited rotatable bonds enhance the drug's bioavailability and stability. By rigorously evaluating and optimizing these properties alongside ADMET criteria, researchers can more effectively identify and refine drug candidates with the highest potential for success in clinical development and eventual therapeutic use.

From the GCMS analysis of Propolis, 44 compounds were identified. These compounds were subjected to the ADMET analysis to predict the drug-likeness properties. Quikprop 4.4 in the Schrodinger and Swiss ADME suite was used to anticipate the activities of biochemical compounds. Drug likeness properties were determined based on Molecular weight, CNS activity, metabolic processes, human oral absorption, percent human oral absorption, brain/blood partition coefficient, dipole movement,

Table 1. Drug-like properties of the selected 13 propolis compounds

Compound	Molecular weight	Q Plog BB	CNS	Human oral absorption %	Human oral absorption %	Lipinski rule
1-[2-(2-Acetoxy-ethoxy)-5-methyl-phenyl]-3-trimethylsilyoxy-2-aza-1,3-butadiene	335.474	-0.786	-1	1	100	0
2,4,4-Trimethyl-5-(1,1,3,3-tetramethylisoindol-2-yl) oxypentan-2-ol	319.486	0.331	1	3	100	0
Trimethylsilyl 3- (trimethylsilyl amino) propanoate	233.457	-0.147	0	3	100	0
Chrysin	254.242	-0.876	-1	3	87.1	0
Ethyl alpha-d-glucopyranoside	208.211	-1.293	-2	2	60.7	0
Quercetin	372.374	-0.075	0	3	100	0
Capric acid	172.26	0.142	--2	3	94	0
Carprofen	284.484	-0.195	-1	2	91.3	1
Tetracontane	563.096	-0.107	2	1	82.7	2
Hexacosane	366.718	1.128	0	2	87.6	1

volume, and total solvent accessible surface (SASA) are among the factors that influence the Lipinski's rule of five and the Jorgensen's rule of three. Out of the 44 compounds, 13 compounds show good drug-like properties. The predicted properties have been evaluated and the drug likeness properties of screened 13 Propolis compounds**.** These molecules obey the standard drug-like properties (Table 1).

Molecular docking: *In silico* molecular docking, analysis is performed against various enzymes that induce PCOS, including Ghrelin, Leptin, and CYP17A. In this docking study, the selected 13 compounds from the ADMET analysis were subjected to further docking studies. From the docking result, the Quercetin and Chrysin molecules show a good binding score compared to the standard drug Lorcaserin (Table 2).

Quercetin with the Ghrelin receptor: In these docking results, the good binding interaction molecules of Quercetin and chrysin with the respective receptor were mentioned and discussed. The 3D and 2D interactions of the Quercetin with the Ghrelin receptor is -9.1 Kcal/mol. The binding score of the Quercetin with The OH group of the Quercetin forms one strong Hydrogen bond with the His 210 residue with 2.39Å bond length (Fig. 1). Further, the aromatic benzene groups are involved in the Alkyl and Pi-Alkyl interactions.

Chrysin with Leptin: The Chrysin molecules show a good binding score with the Leptin receptor. The biding score of the Chrysin with Leptin is -7.1 Kcal/mol. In these binding interactions, the two methoxy and ketone groups forms the three strong hydrogen bond with the Val 123, Ser127, Gln130. The bond length of these interactions is 2.37 Å, 2.08 Å and

Table 2. Molecular docking score of compounds in propolis

Ligand	Ghrelin	Leptin	CYP17A
Quercetin	-9.1	-7.0	-8.3
Chrysin	-8.3	-7.1	-8.2
Lorcaserin	-4.3	-4.8	-6.5

2.57 Å respectively. The Ile 24 amino acid interacted with Quercetin by Pi-Sigma interactions (Fig. 2).

Quercetin with CYP17: The Quercetin molecules show a good binding score compared to the other molecules (-8.3Kcal/mol). In this docking analysis, the active site amino acids of Gly297 and Asp 298 form two strong hydrogen bonds with the methoxy groups and the bond length is 2.4 Å and 2.87 Å respectively. The other active site amino acids Gly301, Ala113, Val482 form Ile 205 Alkyl and Pi-Alkyl interactions with the Quercetin molecules (Fig. 3).

Standard drug Lorcaserin: The Lorcaserin Standard drug shows a binding score of - 4.8Kcal/mol. In this docking analysis, the active site amino acids of Leu 13 in Leptin with aromatic benzyl group and bond length of 4.8 Å with Pi-sigma interaction (Fig. 4a). The Lorcaserin Standard drug shows a binding score of -6.5Kcal/mol. In this docking analysis, the active site amino acids of Leu 66, Phe 42, Pro 44, and PHE 53 with aromatic benzyl group and bond lengths of 4.4 Å, 4.5 Å,4.7 Å,5.0 Å,4.3 Å. The aromatic benzyl group of Lorcaserin form alkyl, Pi-pi stacked, Pi alkyl interactions with the respective amino acids (Fig. 4b). In this docking analysis, the active site amino acids of Asp 3, Phe 4, Ser 6, and Glu 9 with aromatic benzyl group, strong hydrogen bond, and chloride bond with binding lengths of 4.4 Å, 4.5 Å,4.7 Å,5.0 Å,4.3 Å. The aromatic benzyl group and conventional hydrogen bonds of Lorcaserin form Pi-pi, and Carbon hydrogen bonds with the respective amino acids (Fig. 4c).

Molecular dynamic simulation: To analyse the stability of the protein, a molecular dynamic simulation was performed using the best docking score obtained from the Quercetin-Ghrelin complex. The stability of ligands in the aqueous environment of the chosen target's active site and the role of significant amino acids in the interaction between ligand and protein can both be investigated using molecular dynamics (MD) modelling, which is a highly helpful method. When it comes to the Ghrelin receptor, the quercetin molecule has a better docking score than the target receptors in obesity.

The RMSD parameter and the protein-ligand interactions were used to analyse the MD trajectories. The stability and flexibility of molecular docked complexes are discussed in these works. This investigation was carried out following the MM-GBSA computation. The protein-ligand interaction is discussed in this work, and the protein-ligand complex's RMSD fluctuation and variation can be evaluated. If the complex was found to be within the 4Å range, it was considered stable.

MD simulation of Quercetin with the Ghrelin complex for 100 ns in order to assess the stability of the complexes and observe potential ligand binding patterns. The protein complex with the backbone of the quercetin molecule exhibited a shift in RMSD from 0 to 2.34 Å over the first 60 ns (Fig. 5). Additionally, throughout the simulation, the protein configuration's RMSD value increased until, after 58 ns, it stabilised at 2.53 Å. More stability is maintained by the Quercetin molecule in the protein-ligand complex, as evidenced by the average RMSD of the crystal structure complex with the reference molecule of roughly 2.53 Å. Throughout the simulation, the RMSD's variation did not go above 2.4 Å. Additionally, the average 2.61 Å low RMSD values show that the quercetin ligands have established a stable complex during the MD simulation and have attained good equilibrium in the Ghrelin protein's active region.

The Ghrelin complex with Quercetin RMSF graphs is displayed in (Fig. 6). The Ghrelin complex with Quercetin had a mean RMSF value of 1.0 Å. The less flexible residues in the active region are confirmed by the smaller RMSF differential between the ligand and receptor. Based on these findings, it was deduced that quercetin and ghrelin bind together to form stable complexes that do not alter the locations of the active site residues. Although there are minor variations in some residues of Leu 138, Arg178, Arg129, Ala232, and Val181, the overall stability of the complex under study is unaffected by these variations.

Propolis is one of the most significant compounds

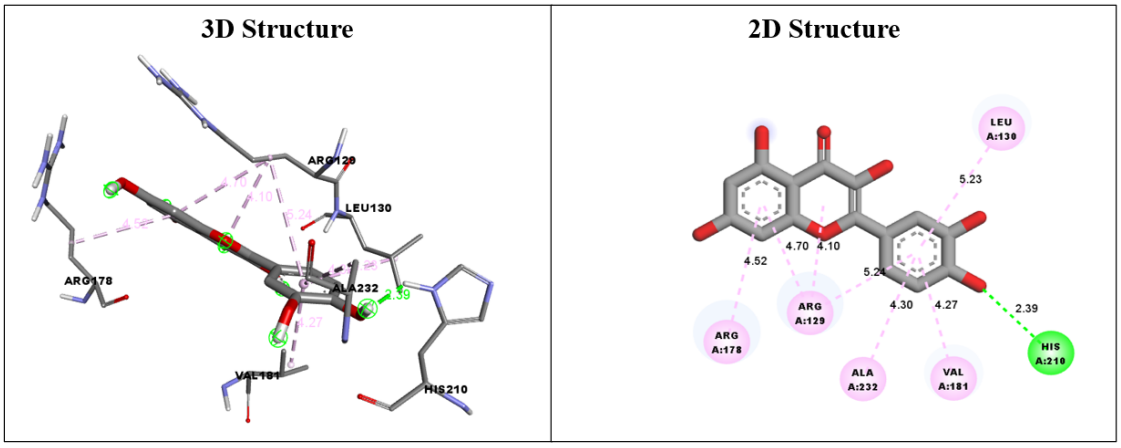

Fig. 1 3D and 2D interactions of the Quercetin with the Ghrelin receptor

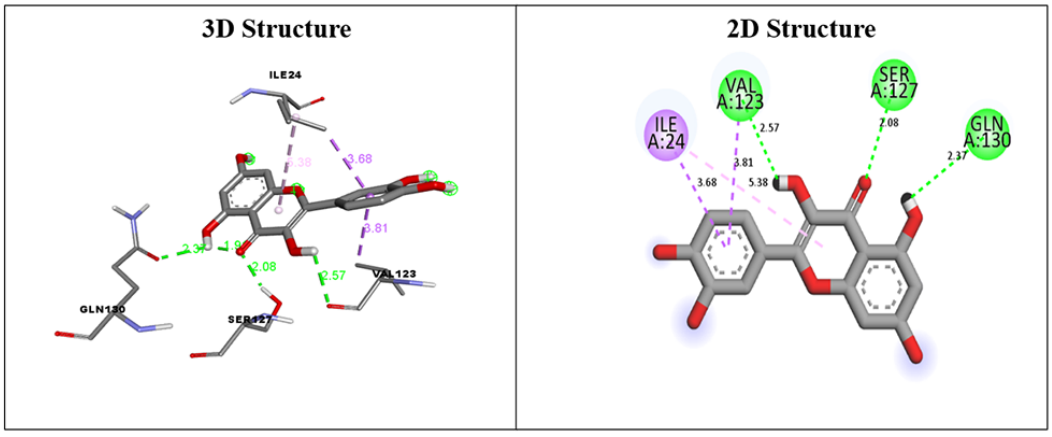

Fig. 2 The 3D and 2D interactions of the Quercetin with the Leptin receptor

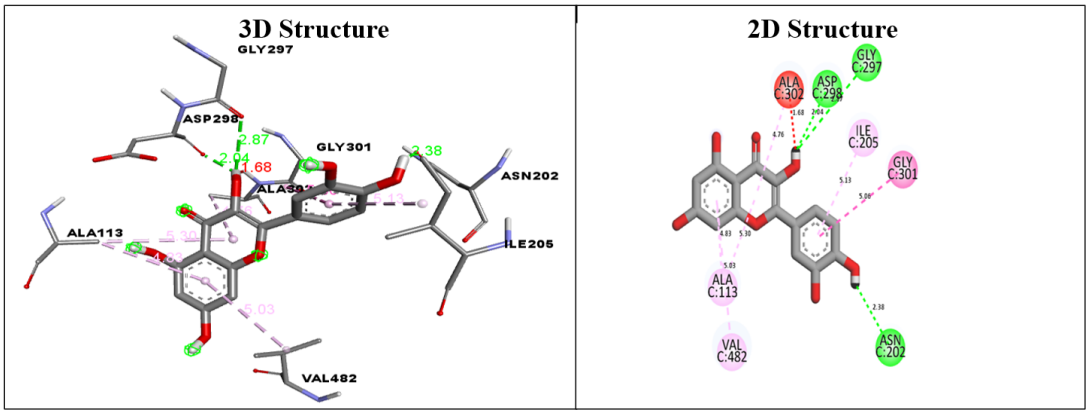

Fig. 3 The 3D and 2D interactions of the Quercetin with the CYP17 receptor

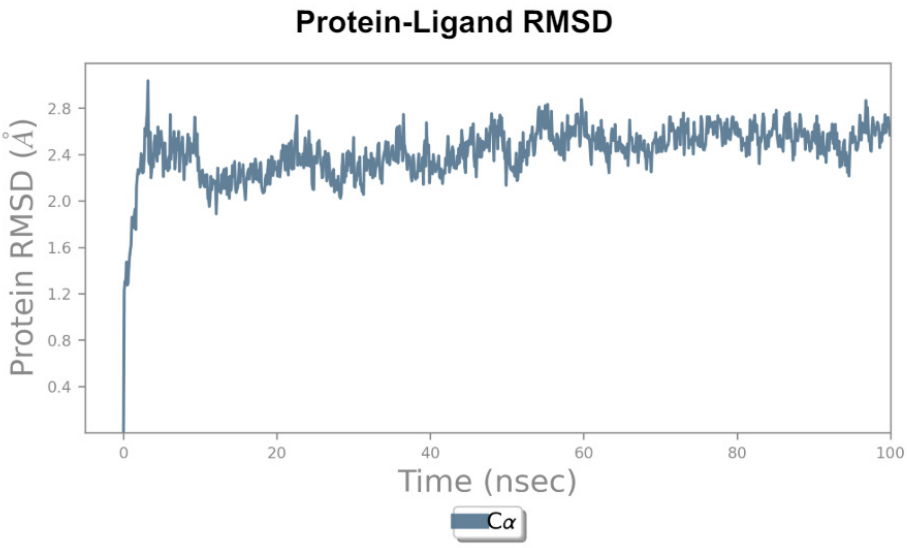

Fig 5. Root mean squared deviation of Quercetin with the Ghrelin complex for 100 ns

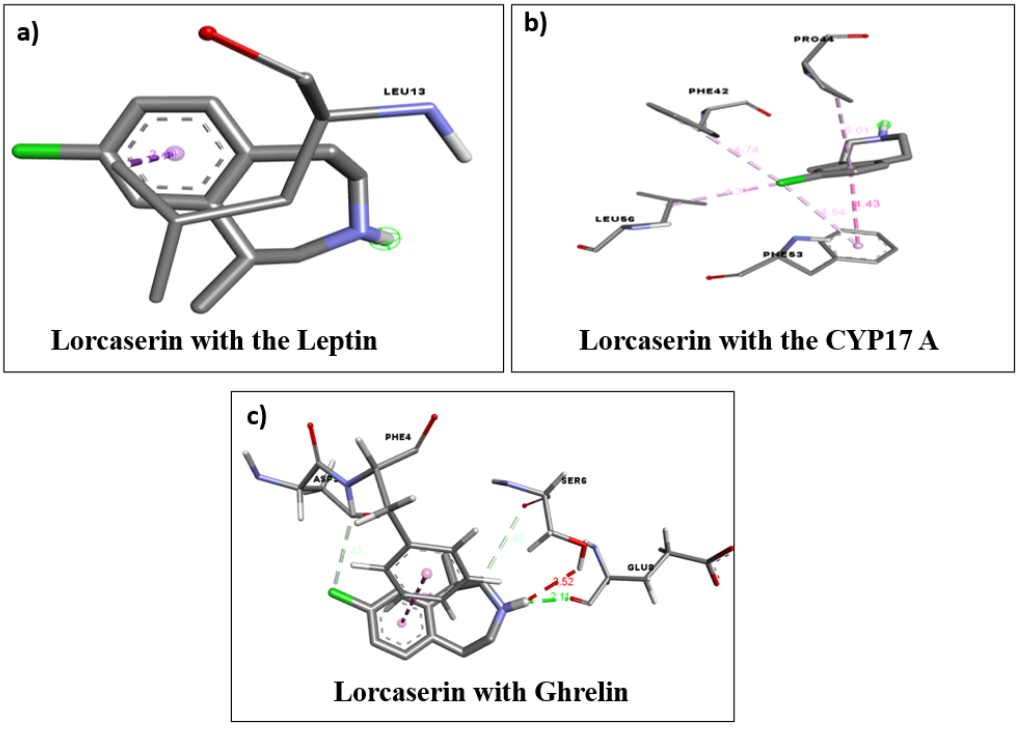

Fig. 4 The 3D binding interactions of Lorcaserin with a) Leptin, b) CYP17A and c) Ghrelin receptor

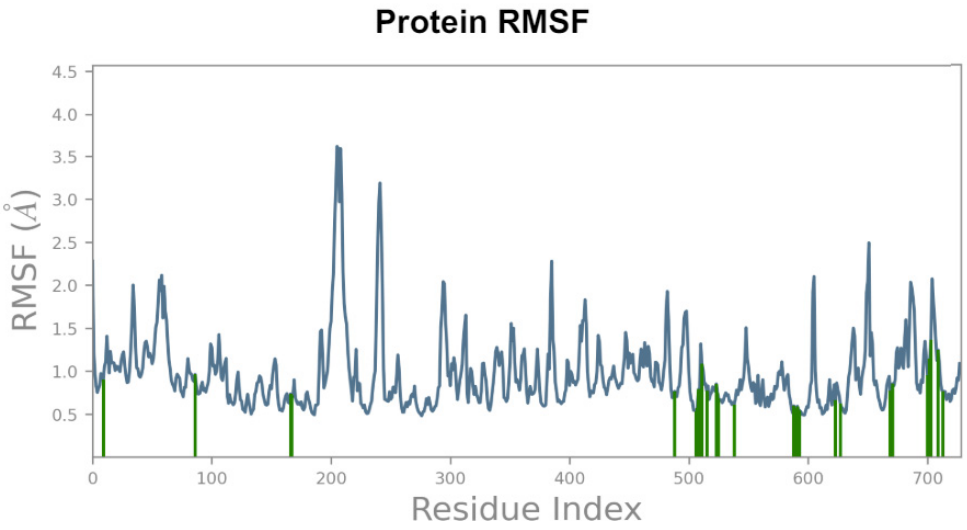

Fig. 6 Root mean squared fluctuation of Quercetin with the Ghrelin complex for 100 ns

with a wide range of biological functions that is produced by various species of honeybees. In this study, ADMET, molecular docking, and molecular dynamics simulations on propolis phytochemicals. Based on the ADMET analysis, screened the best drug-like properties contained in phytochemicals. From the molecular docking analysis, Quercetin and Chrysin ligands as an excellent candidates as inhibitors in Ghrelin, Leptin and CYP17A1 gene receptors comparable with other ligands and standard drugs. The molecular dynamics simulation demonstrates that Quercetin can also serve as good candidates as inhibitors and maintain the ligand-protein (Ghrelin) stability. Further research should be conducted to verify the potency and safety of Quercetin and Chrysin ligands in the treatment of PCOS.

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Description of a new species of genus *Chloroplaga* Hampson (Lepidoptera, Nolidae) from India

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ABSTRACT: A new species of the genus *Chloroplaga* Hampson was identified in Mizoram, India, through detailed morphological and genitalia analysis. This discovery emphasizes the unexplored biodiversity of the region and the necessity of conservation efforts in such ecologically rich habitats.
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KEY WORDS: Morphological, genetalia analysis, Mizoram

INTRODUCTION

India's diverse altitudes and distinct climatic conditions, shaped by the southwest and northeast monsoons, have produced varied ecological niches supporting a wide range of forest types. These include tropical moist deciduous, tropical semi-evergreen, tropical wet evergreen, subtropical, temperate, and alpine forests, each reflecting the country's geographical and climatic diversity. Situated at the intersection of Indian, Malayan, and Indo-Chinese biogeographical zones, India harbours rich biodiversity, including a high proportion of endemic species. The order Lepidoptera, which includes moths, butterflies, and skippers, is globally widespread and it is the third-largest group in the class Insecta. These insects are known for their distinctive scales, covering their wings and bodies, which give them their vivid colours and complex patterns. With membranous wings and a specialised proboscis for feeding, Lepidoptera are among the most successful insect groups in the world, thriving in a variety of environments from deserts to rainforests and mountains.

The family Nolidae, comprising approximately 202 genera and 2179 species (Joshi *et al*., 2021), is dispersed globally, with the highest diversity in tropical regions. There has long been disagreement over the taxonomic classification of its subfamilies, especially Nolinae. While Nolinae was historically treated as part of Noctuidae, it is now recognized as a subfamily within Nolidae based on molecular studies (Zahiri *et al*., 2013). Nolinae moths are small, with distinct forewing patterns and structural features like apodemes on the male eighth tergite. Some members are significant agricultural pests. In this study, a new species of the genus *Chloroplaga* Hampson was discovered in Mizoram, India, an area known for its rich biodiversity. A detailed morphological analysis confirmed its novelty, emphasizing the importance of exploring under-researched regions. Such findings not only expand scientific knowledge but also highlight the critical need for conservation efforts in these biologically rich habitats.

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MATERIALS AND METHODS

In the present study, extensive surveys across Mizoram, India were conducted. Adult Noctuid moths were collected using light traps, including vertical sheets and portable methods (Fry and Waring, 1996), during nighttime. In areas without electricity, Petromax or battery-operated lamps were used. Sampling was conducted during pre-monsoon and post-monsoon seasons. Adult moths collected from the field were immobilized using ethyl acetate vapours and mounted on stretching boards or thermocol sheets. Preserved specimens were stored in airtight wooden boxes lined with thermocol and fumigated with naphthalene balls to prevent pest damage.

Permanent slides of fore and hind wings were prepared following the methods of Common (1970) and Zimmerman (1978) to study venation for accurate identification. Wings were detached using a fine needle, placed in alcohol (70%), descaled with sodium hypochlorite (4% w/v chlorine solution), and washed with distilled water. They were stained 70% with alcoholic eosin for 12–14 hours, dehydrated in graded alcohol, and mounted in Canada balsam. Venation patterns, essential for taxonomic studies, were examined under a stereo zoom microscope. Wing nomenclature followed Comstock and Needham (1898-99), later refined by Imms (1957) and Miller (1970).

For genetallic studies, abdomens were detached with care to avoid damaging genital structures (Robinson, 1976) and softened overnight in 10% KOH to remove muscles and debris. After washing in distilled water and treating with 1% glacial acetic acid to neutralize residual KOH, dissections were performed in 50% alcohol. Male and female genitalia were carefully extracted, with the aedeagus separated and the vesica everted using fine forceps. Structures were dehydrated in graded alcohol, cleared in clove oil, and mounted in Canada balsam on cavity slides. Genitallic terminology followed Klots (1970).

RESULTS AND DISCUSSSION

Genus *Chloroplaga* Hampson

Chloroplaga Hampson, 1912 . *Cat. Lepid. Phalaenae Br. Mus.*: 16: 596.

Type species**:** *Acontia nygmia* Swinhoe.

The genus *Chloroplaga* Hampson (Family, Nolidae) displays a variety of distinct morphological traits. It has a fully developed proboscis, and the palpi are upturned, with the second joint extending to the middle of the frons and slenderly scaled, while the third joint is elongated. The frons are smooth, and the eyes are large and round. In males, the antennae are almost simple. The thorax is covered with a mix of hairs and scales, with no crests present, and the tibiae have slight hair fringes. The abdomen features a dorsal crest only at the base, and males have large lateral scale fans that cover a prominent ventral opening.

The forewings are distinctive, with a pointed and acute apex, an evenly curved, non-crenulate termen, and a lobed inner margin. Venation includes Cu1 and M2 arising near the angle of the cell; R5, R4, R3, and R2 are stalked, with R2 emerging beyond R5, and R1 arising from the cell. The hindwings are characterized by veins Cu1 and M3 originating from the cell angle, M2 extending fully from just above the angle, and M1 and Rs from the upper angle. Additionally, Sc+R1 briefly fuses with the cell near the base, further distinguishing this genus.

***Chloroplaga minuta* sp. nov**.

LSIDurn:lsid:zoobank.org:act:EAFBC97D-5FAA-4012-A8A1-D5606134789E

The head is pale white with upturned, pale brown palpi that have a greenish tinge; the third joint has a pale tip. Antennae are finely ciliated, with a reddish-brown shaft and whitish base. The collar is pale white with a hint of green. The thorax is similarly pale white. The forewings are pale white with a pinkish hue, speckled with green. A green mark appears at the base of the costa and another at the vein's base within the cell. Near the inner margin's base, there is a yellowish patch adjacent to a green speck. A sap-green antemedial patch on the costa merges with a lunulate medial patch in and below the cell, faintly edged in whitish. A small green speck is visible below the costa beyond the middle, while

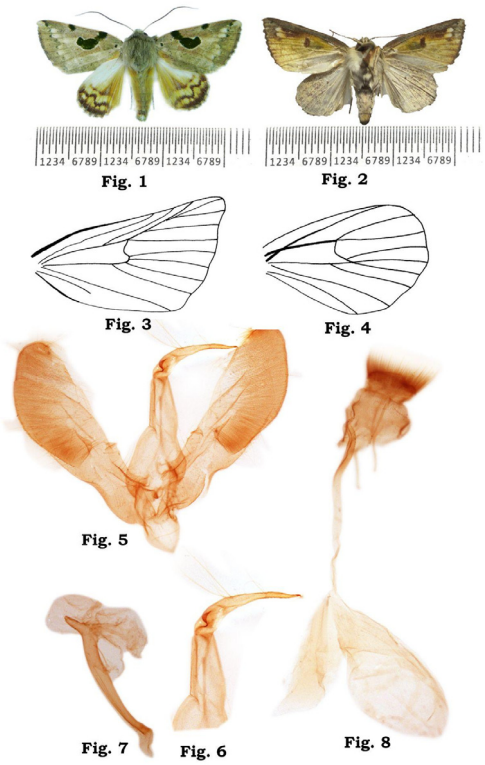

Fig. 1 Upper side & Fig. 2 Under side of *Chloroplaga minuta* **sp. nov.** Adult moth;
Fig. 3 Forewing venation; Fig. 4 Hindwing venation;
Fig. 5 Male genetalia; Fig. 6 Uncus; Fig. 7 Aedeagus;
Fig. 8 Female genetalia

a diffused greenish subterminal waved line extends from a subapical dark brown spot. The cilia are dark brown. The hindwings are yellowish with medial, postmedial, and terminal reddish-brown bands, the medial band showing a sinuous curve in the center. The abdomen is yellowish. On the underside, the forewings are yellowish with whitish costal nervature and inner margins, while the subapical area is suffused with dark green. The hindwings are whitish with reddish-brown speckling.

Male genitalia: The uncus is broad at the base, tapering towards a setose tip that is spined. The tegumen is elongated, narrow, and weakly sclerotized. The juxta is small and round, while the vinculum is V-shaped, small, and also weakly sclerotized. The valve is broad, with a concave and sclerotized saccular margin near the tip. The costal margin is nearly straight, and the tip of the valve is rounded and covered in hair. The aedeagus is long and tubular, narrowing towards the tip and pointed at the base. The vesica is scobinated, and the ductus ejaculatoris enters it subapically.

Female genitalia: The papilla analis is rectangular in shape and covered with hair. The posterior and anterior apophyses are equal in length. The ductus bursae is elongated and becomes sclerotized near the papilla analis. The corpus bursae is also long, narrow, and membranous.

Material examined:

Holotype ♀: Mizoram: Hrangchalkwan 18.ix.2012.

Paratypes: Mizoram: 1 ♀ Champhai 25.ix.2009; 1 ♂, 1 ♀ Hrangchalkwan 18.ix.2012; 1 ♂ Serchip 24.ix.2012; 1 ♂ Sangau 21.ix.2012; 2 ♀♀ Thingsul 18.ix.2009.

The holotype and paratypes are deposited in the Department of Zoology and Environmental Sciences, Punjabi University, Patiala.

Discussion: The genus *Chloroplaga* was first established by Hampson in 1912, with *Acontia nygmia* Swinhoe from Singapore designated as the type species. Warren (1916) later added another species, *C. pallida* Warren, to the genus. Subsequently, Roepke (1948) included *C. javana* Roepke, but this species was later synonymized with *C. pallida* by Holloway (1976). Poole (1989) documented three species under this genus globally: *C. pallida, C. javana,* and *C. nygmia*. Genitalia of *C. Pallida* and *C. nygmia* was described along with pictures by Holloway (2003) in The Moths of Borneo. In the current study, twelve adult specimens of genus *Chloroplaga* Hampson were collected from various localities in Mizoram, India. *Chloroplaga minuta* **sp. nov.** closely resembles *C.pallida* Warren but can be differentiated by distinct male and female genitalic features. In *C. minuta*, the spine on the basal half of the inner valve wall is shorter and less prominent, the proximal end of the aedeagus has a more noticeable curvature, and it tapers to a sharp, well-defined tip. In females, the corpus bursae is reduced in size.

Conversely, in *C. pallida*, the spine on the inner valve wall is longer, the proximal end of the aedeagus is nearly straight with a rounded tip, and the corpus bursae is comparatively larger and more prominent. Based on these differences, *C. minuta* **sp. nov.** is proposed as a new species.

Etymology: The name of the species pertains to a small spine on the valve.

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Efficacy of botanicals and synthetic acaricides against *Aceria noxia* Flechtmann & Tassi, 2020 in vegetable amaranth, *Amaranthus tricolor* L.

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ABSTRACT: Field experiments were carried out to test the efficacy of spiromesifen, fenpyroximate, pongamia oil soap (3%), NSKE (5%), HMO (2%), wettable sulphur and azadirachtin in controlling *Aceria noxia* Flechtmann & Tassi, 2020 (Trombidiformes, Eriophyidae) in amaranthus. Spiromesifen and fenpyroximate were found to be potent miticides and exhibited extended action in managing the mite. Pongamia oil soap and Horticultural Mineral Oil (HMO) showed immediate control of the mite population but failed to show persistence in the field. Although wettable sulphur was effective in reducing mite population, the yellow dust cover over the crop, reduced the acceptance by the farmers. NSKE and azadirachtin, failed to show the complete control over the mites. The reside of fenpyroximate was found to be above the MRL value fixed by FAO. © 2025 Association for Advancement of Entomology

KEY WORDS: Eriophyid, miticides, spiromesifen, fenpyroximate, pongamia oil soap, Horticultural mineral oil, persistence in the field, residue

INTRODUCTION

Vegetable amaranthus (*Amaranthus tricolour*) is a popular leafy vegetable grown in warm temperate and tropical areas. It is grown in India, Myanmar, Malaysia, Taiwan, tropical Africa, Central and South America, South Pacific Islands and the Carribean islands (Tindall, 1983). Amaranthus is a sole leafy vegetable crop, cultivated in the tropical humid region of Kerala (Celine *et al*., 2002). The crop is raised annually in paddy lowlands, homesteads and garden lands in Kerala (Muralikrishna *et al*., 2019). The major factor which hampers yield and productivity of amaranthus is the pest infestation. Leaf webber, tobacco caterpillar and green grasshopper infest the leaves of amaranth (Nair, 1975). Other than insects, mites belonging to Eriophyidae, Tetranychidae etc. are causing

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significant damage on the amaranthus crop worldwide (Dhooria, 2016). The major mites infesting amaranthus crops in Kerala are *Brevipalpus phoenicis* and *Tetranychus* spp. (Binisha and Bhaskar, 2013). Lenin and Bhaskar (2015) observed that amaranthus in greenhouses were also infested by *Tetranychus truncatus*. Flechtmann and Tassi (2020) reported a new eriophyid *Aceria noxia* Flechtmann & Tassi, 2020 (Trombidiformes, Eriophyidae), causing chlorotic patches and deformation in leaves of *Amaranthus viridis* in Brazil.

Eriophyidae is a major family of phytophagous mites. Out of 4600 species of eriophyid distributed worldwide, above 482 species have been reported from India (Bhardwaj *et al*., 2023), with 127 *species* belonging to the *Aceria* genus (Huang, 2008). However, no reports of Eriophyidae in amaranthus were confirmed in India until first incidence was reported in Kasaragod district of Kerala in the summer months of April and May 2019. The infested amaranthus had crinkled, miniatures and malformed leaves. The plants appeared stunted in growth and shoots were seen fibrous after cutting for culinary purpose. The incidence reduced the number of harvests to at least 50 per cent and there was more than 50 per cent reduction in revenue as surveyed by the farmers of the affected area. Marketability was found to be affected due to the fibrous nature of leafy plants (Sreekumar *et al*., 2019). Later it is confirmed that the species is *A. noxia* itself. So, different acaricides were tested for the management of this pest which is described in this paper.

MATERIALS AND METHODS

a. Preparation of pongamia oil soap: Mixed 55g of caustic soda with distilled water (100 ml) and left with no disturbance for 4 hours. The solution homogenized into the solution prepared with 55g soap stone power and pongamia oil (1 litre). The prepared pongamia oil soap solution was then kept for solidification.

b. Preparation of 'Neem Seed Kernel Extract' (NSKE 5%): The 50g seed kernel powder was kept in single muslin cloth bags and were immersed in 1 liter of water overnight. Then bag was squeezed and taken out. The suspension with kernel extract was made up to 1 liter and used for further studies.

Experiments were carried out with farmer's variety in farmer's plots at Thaikadappuram, Kanhangad, Kasaragod district in Randomized Block Design with 8 treatments (Spiromesifen 22.9 SC @96g ai ha^{-1}, Fenpyroximate 5 EC @15g ai ha^{-1}, Pongamia oil soap (3%), NSKE (5%), Horticultural Mineral Oil (2%), Wettable sulphur 80 WP 1600g ai ha^{-1}, Azadirachtin 3000ppm and Absolute control), and 3 replications. Application of treatments were done two times. The evaluation was carried out on severely infested plants during May and June 2019. A single treatment plot consisted of a row of 15 plants. Observations were made one day before treatment and one, three, five, seven and fourteen days after application. The top five leaf axils from three plants of each row were examined on each observation and the number of mites were counted under the stereomicroscope. The statistical analysis of data was carried out using Analysis of Variance (ANOVA). The significance between treatments was compared using 'Web Agri Stat Package' (WASP 2.0) software. To compute a per cent reduction in mite population, Henderson and Tilton formula was utilized (Henderson and Tilton, 1955).

$$C_{\text{corrected per cent}} = \left(1 - \frac{n \text{ in Co before treatment} \times n \text{ in T after treatment}}{n \text{ in Co after treatment} \times n \text{ in T before treatment}}\right) \times 100$$

Where, n = insect population, T = treated, Co = control

Residue analysis: The shoot samples sprayed with Spiromesifen and Fenpyroximate collected during the second application (on the fifth, seventh and tenth day) were analysed at the Pesticide Residue and Analytical Laboratory, AINP on Pesticide Residue, College of Agriculture, Vellayani. The reference method followed was QuEChERS –AINPPRKAU/MOA-02 (AOAC 20th Edition 2016: Chapter 10, 2007.01) and 'European Union reference laboratory multi residue method' for fruits and vegetables using QuEChERS (GC-ECD & FPD, GC-MS) (Anonymous, 2007).

RESULTS AND DISCUSSION

Mite population in different treatments:

Table 1. Average population of Aceria mites in top five leaf axils per shoot recorded during the experiment

Treatments	First application of treatments						Second application of treatments					
	1 DBA	1 DAA	3 DAA	5 DAA	7 DAA	14 DAA	1 DBA	1 DAA	3 DAA	5 DAA	7 DAA	14 DAA
Spiromesifen 22.9 SC @ 96g ai ha^{-1}	244.000 (15.599)	0.000 (0.707)^{d}	4.000 (2.114)^{d}	5.777 (2.401)^{e}	9.223 (3.033)^{f}	20.557 (4.519)^{d}	163.110 (12.753)	0.000 (0.707)^{d}	1.443 (1.189)^{e}	5.110 (2.231)^{e}	8.777 (2.957)^{ef}	11.890 (3.437)^{e}
Fenpyroximate 5 EC @ 15g ai ha^{-1}	277.110 (16.636)	0.000 (0.707)^{d}	0.223 (0.832)^{e}	2.557 (1.585)^{f}	6.000 (2.445)^{g}	13.553 (3.675)^{d}	163.110 (12.753)	0.000 (0.707)^{d}	1.557 (1.202)^{e}	5.223 (2.284)^{e}	7.113 (2.666)^{f}	17.667 (4.202)^{d}
Pongamia oil soap (3%)	276.667 (16.629)	0.000 (0.707)^{d}	4.223 (2.171)^{d}	6.667 (2.575)^{e}	10.557 (3.245)^{ef}	20.777 (4.554)^{d}	165.890 (12.870)	0.000 (0.707)^{d}	5.330 (2.302)^{d}	7.890 (2.809)^{d}	12.000 (3.464)^{d}	16.000 (3.992)^{d}
NSKE 5%	278.333 (16.676)	35.890 (6.018)^{b}	42.220 (6.532)^{b}	51.557 (7.178)^{b}	64.557 (8.033)^{b}	146.997 (12.123)^{b}	176.557 (13.286)	15.447 (3.974)^{b}	27.223 (5.213)^{b}	40.447 (6.355)^{b}	55.780 (7.466)^{b}	76.333 (8.735)^{b}
HMO (2%)	276.223 (16.620)	0.000 (0.707^{d}	3.000 (1.865)^{d}	7.557 (2.746)^{de}	11.780 (3.432)^{de}	27.110 (5.199)^{cd}	164.220 (12.809)	0.000 (0.707)^{d}	1.880 (1.320)^{e}	5.110 (2.231)^{e}	8.553 (2.015)^{ef}	17.447 (4.175)^{d}
Wettable Sulphur 80WP	278.553 (16.687)	0.000 (0.707^{d}	4.223 (2.171)^{d}	9.443 (3.068)^{d}	13.000 (3.604)^{d}	27.557 (5.247)^{cd}	157.557 (12.551)	0.000 (0.707)^{d}	2.333 (1.510)^{e}	4.333 (2.075)^{e}	10.443 (3.232)^{de}	17.667 (4.198)^{d}
Azadirachtin 3000ppm	285.553 (16.892)	21.777 (4.717)^{c}	29.89 (5.511)^{c}	43.223 (6.569)^{c}	56.000 (7.483)^{c}	70.333 (7.699)^{c}	155.557 (12.470)	8.330 (2.972)^{c}	14.777 (3.843)^{c}	23.777 (4.867)^{c}	33.417 (5.765)^{c}	56.890 (7.542)^{c}
Control (Water spray)	267.337 (16.350)	273.110 (16.541)^{a}	278.557 (16.705)^{a}	284.887 (16.878)^{a}	289.887 (17.026)^{a}	324.223 (18.005)^{a}	160.447 (12.663)	165.000 (12.663)^{a}	197.447 (14.051)^{a}	227.557 (15.083)^{a}	256.223 (16.006)^{a}	299.443 (17.304)^{a}
CD (0.05)	NS	5.262 (0.374)	3.015 (0.335)	5.288 (0.423)	4.138 (0.282)	33.693 (2.526)	NS	4.668 (0.331)	3.472 (0.494)	6.423 (0.485)	5.853 (0.454)	4.707 (0.379)

*In a colum means followed by diffrent letters are statistically significant; Values in parentheses are arc sin values

Uniform population (244.00 to 285.55 mites) was observed in top five leaf axils per shoot, a day before the first application. The plots sprayed with acaricides, pongamia oil, HMO and wettable Sulphur recorded no population of mite a day after the spray. However, mites were seen in plots sprayed with azadirachtin (21.777 mites) and NSKE (35.890 mites), both were statistically different (Table 1).

The third day observation recorded fenpyroximate (0.223 mites) as the superior treatment, followed by HMO @ 2 per cent (3 mites) and spiromesifen (4 mites). After five days of application, fenpyroximate recorded the least population (2.557 mites), significantly different to other treatments. This was followed by spiromesifen, pongamia oil soap, HMO and wettable sulphur, with average population of 5.777, 6.667, 7.557 and 9.443 mites, respectively and were on par to each other. A week after the spray, fenpyroximate showed the least population of six mites. The next best result was given by spiromesifen sprayed plots with 9.223 mites. Other treatments, pongamia oil soap, HMO, wettable sulphur recorded significantly similar populations of 10.557, 11.780 and 13.000 mites respectively. Two weeks after the spray, fenpyroximate maintained the dominance among the treatments in controlling the population (13.553 mites). This was followed by the statistically similar results of spiromesifen, pongamia oil soap, HMO, wettable sulphur and azadirachtin, with a population mean of 20.557, 20.777, 27.110, 27.557 and 70.333 mites respectively.

During second application, a uniform population in the range of 160.447 to 176.557 mites were observed in the field, a day before the spray. On the first day after the application there was complete absence of mites on plants treated with acaricides, pongamia oil, HMO and wettable sulphur. On the third day, the least mean population was seen in plants sprayed with spiromesifen (1.443 mites), succeeded by fenpyroximate sprayed plots (1.557 mites). Other acaricides, which are on par to the former were HMO and wettable sulphur, with mean population of 1.880 and 2.333 mites, respectively. On the fifth day, wettable sulphur showed

maximum effectiveness in managing the population (4.333 mites). Spiromesifen, HMO, fenpyroximate showed statistically similar results with 5.110, 5.110 and 5.223 mites respectively. On the seventh day, population was lowest in fenpyroximate sprayed plants (7.113 mites). This was statistically similar with the results of spiromesifen and HMO with respective mean populations of 8.777 and 8.553 mites. Wettable sulphur (10.443 mites) and pongamia oil soap (12.000 mites) recorded the next best treatment. Two weeks after the treatment, the lowest count was observed in the plot sprayed with spiromesifen (11.890 mites). An average population of 17.447, 16.000, 17.667 and 17.667 mites were observed in plots sprayed with HMO, pongamia oil soap, fenpyroximate and wettable sulphur, respectively.

Percentage reduction in mite population:
Cent per cent reduction was observed after the first day of spray of acaricides in both rounds of application. NSKE and azadirachtin, achieved 92.460 and 86.970 per cent reduction, respectively (Table 2).

The third day witnessed fenpyroximate as the superior treatment, with a reduction per cent of 99.93, followed by HMO (99.86 %), pongamia oil soap (98.58 %), wettable sulphur (98.50 %), spiromesifen (98.32 %), azadirachtin (89.83 %) and NSKE (85.13 %). Fenpyroximate sprayed plots recorded 99.02 per cent reduction on the fifth day, followed by spiromesifen (97.72%) and pongamia oil soap (97.65%).

The next best treatments were HMO (97.41%) and wettable sulphur (96.77%). A reduction was observed in azadirachtin (85.69%) and NSKE (82.43%) sprayed plots. After a week, fenpyroximate again proved the best treatment with a per cent reduction of 98.01. The oils, pongamia oil soap and HMO showed 96.51 and 95.98 per cent reduction of mites respectively. Spiromesifen sprayed plots reduced the population by 95.77 per cent. The least efficient treatments were botanicals, azadirachtin (81.84%) and NSKE (78.33%). Two weeks after the spray, highest reduction was observed in fenpyroximate applied plots (95.97%). This was succeeded by pongamia oil soap and spiromesifen, with respective population reduction of 93.81 and 92.70 per cent. The former observations were on par with results of fenpyroximate. Statistically similar results were observed in plots sprayed with HMO (91.80%) and wettable sulphur (91.74%). NSKE recorded 56.05 per cent reduction.

The first day of observation of the second application, both azadirachtin and NSKE had the lesser efficiency of 94.74 and 91.42 per cent. On the third day, maximum per cent reduction was observed in spiromesifen (99.32%), followed by on par results of fenpyroximate (99.13%), HMO (99.07%) and wettable sulphur (98.76%). On the fifth day, wettable sulphur showed the highest reduction of 97.84 per cent. HMO, spiromesifen and fenpyroximate had the next best reduction per cent (97.68, 97.64 and 97.57%). The less effective ones were azadirachtin and NSKE (with 88.94 and 83.41%). A week after the spray, fenpyroximate showed the highest percentage of reduction (97.09%), followed by HMO (96.64%), spiromesifen (96.34%), wettable sulphur (95.55%) and pongamia oil soap (95.34%). Two weeks of observation, spiromesifen, pongamia oil soap, HMO, fenpyroximate and wettable sulphur were statistically similar in results (95.89, 94.65, 94.09, 93.85 and 93.75% respectively). Significant variant results in NSKE (76.60%) and azadirachtin (79.97%) were observed.

The results obtained from the study clearly revealed fenpyroximate has the best miticidal action on all the respective days of observation in the first season. In the second application, it proved the best treatment after spiromesifen, which was on par with the former on the third, fifth and seventh days after treatment. This is similar to the results from the pesticide evaluation studies of Azevedo *et al*. (2013) against *A. litchii*, in which fenpyroximate recorded the highest mortality followed by sulfur, abamectin and hexythiazox. Spiromesifen, evidenced as the best treatment on all days, after fenpyroximate during first application. The second application revealed the highest control of spiromesifen on the fifth and seventh observation days. This is like the findings of Suganthi *et al*. (2006), in which

Table 2 Percentage reduction in Aceria mite population on amaranthus after the application of acaricides

Treatments	First application, reduction% - in DAA					Second application, reduction% - in DAA				
	1	3	5	7	14	1	3	5	7	14
Spiromesifen	100[a]	98.32[b]	97.72[b]	95.77[b]	92.70[ab]	100[a]	99.32[a]	97.64[a]	96.34[a]	95.89[a]
Fenpyroximate	100[a]	99.93[a]	99.02[a]	98.01[a]	95.97[a]	100[a]	99.13[a]	97.57[a]	97.09[a]	93.87[a]
Pongamia oil soap	100[a]	98.58[b]	97.65[b]	96.51[b]	93.81[ab]	100[a]	97.32[b]	96.49[a]	95.34[a]	94.65[a]
NSKE	86.97[c]	85.13[d]	82.43[e]	78.33[d]	56.05[d]	91.42[c]	87.23[d]	83.41[c]	79.89[c]	76.60[c]
HMO	100[a]	98.86[ab]	97.41[bc]	95.98[b]	91.80[b]	100[a]	99.07[a]	97.68[a]	96.64[a]	94.09[a]
Wettable Sulphur	100[a]	98.50[b]	96.77[c]	95.64[b]	91.74[b]	100[a]	98.76[ab]	97.84[a]	95.55[a]	93.75[a]
Azadirachtin	92.46[b]	89.83[c]	85.69[d]	81.84[c]	71.08[c]	94.74[b]	92.15[c]	88.94[b]	84.94[b]	79.97[b]
Control	0.00[d]	0.00[e]	0.00[f]	0.00[e]	0.00[e]	0.00 [d]	0.00[e]	0.00[d]	0.00[d]	0.00[d]
CD (0.05)	2.32	1.20	0.73	1.43	4.03	1.51	1.48	2.38	2.18	2.29

*In a colum means followed by diffrent letters are statistically significant; Values in parentheses are arc sin values

spiromesifen was found to be superior to triazophos in controlling coconut *Aceria* mite, and reduced population to 17.89 per cent, on the seventh day. The translaminar action of spiromesifen was superior in its action among nine miticides tested against aloe mite, *A. aloinis*, and can be recommended as a treatment either alone or along with cultural methods (Villavicencio *et al*., 2014).

Pongamia oil soap also proved good control on the mite population and can be used as one of the best options for organic farmers. The oil soap brought the control of mite to 96.51 per cent, a week after the spray. This is different from the observations of Begum *et al*. (2013), in which pongamia leaf extract and ginger extract tested the least effective plant products among neem leaf extract, neem oil, azadirachtin, neem oil based azadirachtin, garlic extract in controlling coconut perianth mite, *A. guerreronis* Keifer.

By the seventh day of treatment, HMO reduced population (95.98% in the first application and 96.64% in the second application). This is similar to the subsequent reduction in population of pink citrus rust mite, *Aculops pelekassi* (Keifer) from the first to the third spray, with HMO only foliar spray programs in selected locations of Florida (Childers, 2011). Wettable sulphur was also a better treatment when compared to all the respective treatments except botanicals, which did not fail to reduce the pest population. The drawback of this treatment was the white dusty appearance on the leaves, which will deter the consumers. This was on par with the best treatment of the season. According to Navik *et al*. (2015), wettable sulphur reduced *Calepitrimerus azadirachtae* on neem, by 49.61 per cent on the third day of spray.

Azadirachtin recorded population reduction (81.84 and 71.08% in the seventh and fourteenth days of observation tespectively). This is against to the findings of Bagde *et al*. (2014), who observed that neemazal 5 per cent spray, along with root feeding, reduced *A. guerreronis* by 23.43 and 38.07 per cent over control after one and two weeks of time. Both the botanicals, NSKE and azadirachtin never met the need, and the mite population were on increasing direction. Botanicals, as of the previous season, failed to meet the target and the plant counted a greater number of live mites, compared to other treatments during second treatment. This is against the findings of Pushpa and Nandihalli (2008), which stated NSKE 5 per cent as the superior one among neem oil and neemazal in controlling population of coconut perinath mite, *A. guerreronis*.

Residue analysis: The analysis detected fenpyroximate residues on an average 0.29 mg kg^{-1}, 0.30 mg $kg^{-1}$and 0.22 mg kg^{-1} in the cuttings collected after 5, 7 and 10th days after treatment. The MRL value fixed by European Union and FAO is 0.01mg kg^{-1}. The residue of spiromesifen was

found to be in BQL (Below Quantitative Level) in which the LOQ (Limit of Quantification) is 0.01 mg kg^{-1}

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Effectiveness of plant essential oil nanoemulsions in controlling, *Leptocorisa oratoria* (Hemiptera, Alydidae) in lab and field conditions

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ABSTRACT: Formulated basil and turmeric essential oil nanoemulsions were evaluated for their effect on eggs and adults of *Leptocorisa oratoria* (Fabricius, 1764) (Hemiptera, Alydidae), pest of rice. Basil and turmeric nanoemulsions of 97.33±52.13 and 42.39± 10.76 d. nm were prepared and characterized. The ovicidal assay showed that the basil oil nanoemulsion has an LC_{50} of 13724 and the turmeric oil nanoemulsion has 14337ppm. The laboratory evaluation of basil oil nanoemulsion on adults showed an LC_{50} of 12187ppm. Turmeric oil nanoemulsion showed a delayed toxicity effect. Acid and alkaline phosphatases were higher in the concentrations of nanoemulsion formulations than in the control. Carbohydrate varied with both formulations. In pot culture and field count, the insect number was found to be affected significantly in the treatments. This study revealed the impact of basil and turmeric essential oil nanoemulsions on *L. oratoria* eggs and adults. © 2025 Association for Advancement of Entomology

KEY WORDS: Paddy, gundhi bug, sucking pest, basil, turmeric, eggs, toxicity

INTRODUCTION

Rice is the primary staple food worldwide, mainly used in Asian countries (Hassan *et al.,* 2022). Major factor affecting paddy cultivation is, the insect pest incidents in their different stages of growth. Insect pests in rice start from their early seedling stage to the late maturation stage. Each stage is important because pest incidence in either stage can reduce the yield drastically. However, from flowering to grain maturation, any damage can reduce the yield considerably. *Leptocorisa oratoria* (Fabricius, 1764) (Hemiptera, Alydidae), is a significant pest of rice that attack the rice by sucking the sap from the milky grain (Hosamani *et al*., 2009). High losses have been reported in India (Litsinger *et al*., 2015). The rice grains became chaffy and smelly due to their secretions, reducing the yield of rice. Plant-based pesticides have been found to be an excellent alternative to chemical pesticides. Plants such as neem, basil, pyrethrum and tobacco have insecticidal activities (Roark, 1947). Plant essential oils have also been found to be effective in controlling pests. The major drawbacks of essential oils are that they evaporate easily and lack permeability. By overriding these problems, essential oils can act as good insecticides.

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Nanotechnology is a new emerging area of science, especially in medicine and cosmetics. The size of molecules enables them to enter the body quickly. Nanoemulsions are one of the major areas in nanotechnology. Nanoemulsions are nanosized micelles of 20-700 nm of size (Jaiswal *et al.,* 2015; Mahdi *et al.,* 2006; Sharma *et al*., 2010; Silva *et al.,* 2012). The micellar formation traps the oils inside the surfactant. With nanometric size, the emulsions can easily enter the body and are less toxic. Nanoemulsions can also override the problems of emulsions such as creaming, coalescence and flocculation. Preparing essential oil nanoemulsions can prevent the problems of essential oils as insecticides. Basil and turmeric are known for their excellent insecticidal activity. This study aimed to prepare nanoemulsions of commercially available essential oils of basil and turmeric of appropriate size. And to evaluate of basil and turmeric nanoemulsions effect in rice pest *L. oratoria* eggs in lab conditions and their effect on adults *L. oratoria* in the lab, pot culture and field conditions.

MATERIALS AND METHODS

Leptocorisa oratoria were reared according to Heinrichs *et al.* (1981) with minor variations. Briefly, the adult insects of both sexes were collected from paddy fields of Velur, Thrissur, Kerala (10.645^0N; 76.170^0E), and transferred to the cages of 40x40x60cm containing rice plants with panicles in milky grain stage. Insects were identified with the keys (Ahmed, 1965; Dolling, 2006). Fresh rice plants with milky grain panicles were provided in cages every week. Eggs were collected by cutting the leaves and placed in a plastic cup with water in the base to avoid the shrinking of leaves. When the nymphs arrived, they were transferred to the abovementioned rearing cages. The first-generation adults were used to check adulticidal activity and their eggs for ovicidal assay. Essential oils of basil and turmeric were purchased from Synthite Industries, Kadayirippu. Tween 80 (Merk) was used as the surfactant. All preparations were done with distilled water. Malathion 50 EC was purchased from the local market. All the chemicals used for biochemical analysis were analytical grade.

GC-MS (Shimadzu GC-MS, Model Number QP2010S) analysis was carried out to detect the components of essential oils. Helium gas was used as the carrier gas at the rate of 1mL/minute with a split ratio of 1:20. Temperatures of 60^oC and 280^oC were the injector temperature and detector temperature. Mass spectra were recorded over the 50 – 500 m/z range using EI mode at 70 eV. NIST 11 and Wiley 8 libraries were used for Mass spectrum analysis.

According to Ghosh *et al.* (2013), nanoemulsions of basil and turmeric oils were prepared and characterized with some variations. In all the preparations, the concentration of oils was kept at 6 per cent. Oil and surfactant Tween 80 were taken in four ratios: 1:1, 1:2, 1:3 and 1:4. Initially, oil and surfactant were mixed using a magnetic stirrer at 800 rpm for 15 minutes. This mixture was then added to water under magnetic stirring at 800 rpm dropwise until the emulsion formed, and then the mixture was mixed for 30 minutes. Using an ultrasonicator (Pci Analytics – PKS- 750 FM), this primary emulsion was sonicated for 10- 30 minutes according to the appearance. pH and electrical conductivity were checked using a pH meter and a conductivity meter; transmittance was checked using an Antech UV-Visible spectrophotometer (St. Thomas College, Thrissur); particle size and zeta potential were checked using a Malvern Zetiser from Sree Chitra Thirunal Institute for Medical Sciences and Technology, Thiruvananthapuram. Stability was checked by centrifuging at 7800 rpm for 30 minutes in the Eppendorf centrifuge 5430 R. The stable nanoemulsions were then further selected for further ovicidal and adulticidal assay.

The toxicity of nanoemulsions on eggs was determined by the dipping method. The eggs were collected by cutting the leaves of rice plants. Each batch of eggs was dipped in different concentrations of nanoemulsions such as 60000, 50000, 20000, 10000 and 5000ppm, distilled water as control, and malathion as positive control. The eggs were observed for seven days for hatching. The number of hatched and unhatched eggs was counted. The eggs did not hatch after seven days and were considered dead. The hatching rate, inhibition rate

and Effective inhibition rate index (EII) were calculated using the formulas given below (Moungthipmalai *et al.,* 2023).

Hatching rate % = (NE/NT) x 100

NE – total number of hatched eggs; NT – Total number of eggs

Inhibition rate % = 100 – hatching rate%

Effective Inhibition rate Index (EII) = % Inhibition rate of each formulation/ % Inhibition rate of PC

EII <1 - Formulation was ineffective as malathion, EII = 1 – Formulation was as effective as malathion.

The effect of nanoemulsions on adult *Leptocorisa* was studied in laboratory conditions. Different concentrations of nanoemulsions ranging from 60000, 50000, 20000, 10000 and 5000ppm were used for the study. Distilled water was used as a control and malathion was used as a positive control. Each treatment consists of 25 adult insects with five replicates. Twenty-five adult insects with rice panicles on milky grain stage in 500mL plastic jars were covered by small mesh. Different concentrations of nanoemulsions, control, and positive control were sprayed onto the jar through the mesh. The mortality rate was recorded after each day up to seven days. The dead insects were stored in a deep freezer for further biochemical analysis.

According to Dua and Viniti (2013), biochemical analyses, such as carbohydrates (Anthrone reagent method) and acid and alkaline phosphatases, were determined to verify the effect of nanoemulsions on insects.

The effective concentrations of the nanoemulsions in the laboratory study were further evaluated in pot culture. In the pot culture study, the insects were released into a cage of 40x40x60cm containing rice plants in the milky grain stage. These rice plants were free from other insects. Then, 20 adult bugs were released into each cage. After the bugs were released, the cage was sprayed with respective formulations along with control and positive control with three replicates. The number of dead insects was recorded at 3, 5, 7, 10, 15 and 20 days after the treatment.

The effective concentrations from the pot culture study were further evaluated in field conditions according to Heinrich *et al.* (1981) with minor variations. Field study was conducted at Velur, Thrissur, Kerala, during the agriculture season 2022-23 from September to December. For the field study, 28 days of "Uma" rice seedlings were transplanted to a field of 4x4m plots under irrigated conditions. A total of 12 plots were used for the study. The selected concentrations of basil oil nanoemulsion and turmeric oil nanoemulsions, along with water as control and malathion as positive control, were sprayed when the gundhi bug population appeared at the economic threshold level using a hand-held sprayer in the fields. After the first spraying, one more spraying was done on the 14th day. Numbers of insects in the fields were counted in triplicate from a 1 m^2 area from each treatment before the first spraying, 5, 10, 15 and 20 days after spraying the insecticides. The data were analyzed using SPSS version 25. For the ovicidal assay, one–way ANOVA was used to compare mean value, hatching rate, inhibition rate, and effective inhibition rate index among different concentrations of insecticides. The LC_{50} value after 48 hours of treatment was calculated using probit analysis.

RESULTS AND DISCUSSIONS

GC-MS analysis of Oil samples: GC-MS analysis of Basil oil showed two major peaks in the chromatogram, 55.92 per cent estragole with RI 16.803 (peak 4) and 37.62 per cent linalool with RI 12.494 (peak 2) as the major compounds. Other compounds, such as menthol, cineole, alpha, and beta-caryophyllene, were also detected (Fig. 1). Ten compounds were detected in turmeric oil. Ar-turmerone was the primary compound (see peak 9, with 63.45% area, 35.96 RI). Curlone (see peak 10, with 9.12% area, 37.22 RI), alpha-zingiberene (see peak 5, with 7.23% area, 29.28 RI), alpha–curcumin (see peak 4, with 6.22% area, 28.77 RI), and turmerone etc., were also detected (Fig. 2).

Nanoemulsions were prepared using nonionic

surfactant Tween 80 due to its high hydrophilic-lipophilic value (15), long-term stability, and low toxicity; the ultrasonication method was adopted for smaller nanoemulsions. Two nanoemulsion formulations were developed from each oil, such as B2 and T4 (Table 1). The compositions B2 (1:2) and T4 (1:4) were found to be more stable than other formulations after characterization. B2 and T4 showed smaller sizes of 97.33±52.12 and 42.39± 10.76 d. nm, PDI of 1.19±1.86 and 0.38±0.16 and zeta potential of -1.01±0.36 and -0.48±0.16 mV. Centrifugation tests showed that B2 and T4 were stable without a phase separation. These stable nanoemulsions were further studied for toxicology.

Ovicidal Assay: The ovicidal assay showed that 60000ppm of both Basil oil and Turmeric oil nanoemulsions have a hatching rate of zero, implying that both have a high inhibition rate of 100 percent. Effective Inhibition rate Index of 60000ppm was found to be close to 1, indicating that this formulation was as effective as the positive control, malathion. 5000 ppm showed the least inhibition rate of 7.33±3.05 and 11.33±2.30 in basil and turmeric nanoemulsions (Table 2). LC_{50} values were found to be 13724 and 14337ppm for basil and turmeric oil nanoemulsions respectively (Table 3).

Adulticidal assay: Basil oil showed a higher mortality rate at 24 hours than turmeric oil. At 60000ppm, mortality was 89.25 per cent for basil oil, while turmeric oil showed only 27.27 percent mortality. At 48 hours, the mortality rate for turmeric oil nanoemulsions was 100 per cent for 60000, 50000 and 20000ppm (Fig. 3). In the case of basil oil, only 60000ppm showed 100 per cent mortality at 48 hours. LC_{50} of basil oil was 12187ppm (LL 6782 – UL 18526) at 48 hours, with regression equation y = -0.221+3.060x and χ^2 27.073(DF3),

Biochemical analysis: Total carbohydrate values varied with basil and turmeric oil nanoemulsions (Fig. 4). For basil nanoemulsions, the total carbohydrate value decreased with increasing concentrations. Turmeric oil nanoemulsion was found to have increasing concentrations of total carbohydrates with increasing concentrations of turmeric nanoemulsions. Both acid and alkaline phosphatase values were found to be higher with high concentrations of both nanoemulsions compared with control (Fig. 5, 6).

Pot culture experiment: The nanoemulsions of both evaluated in the pot culture experiment showed 78 percent mortality on the 3rd day and 100 percent mortality on the 20th day by the basil oil at 60000ppm. Turmeric oil at 60000ppm showed 81 percent mortality on the 3rd day and 95 percent mortality on the 20th day. Nanoemulsions of basil oil at 5000 ppm showed 91 percent mortality after 20 days of treatment. However, turmeric oil had higher mortality than basil nanoemulsion at 3 days after treatment of 70 percent. However, the effectiveness was found to be higher for basil than turmeric (Table 4).

Field experiment: In the field study, the insect was visually counted before treatment and 5, 10, 15 and 20 days after treatment (Table 5). In control, the pre- count 11.67±2.08 m^{-2} increased to 30.33±1.53 after 20 days of treatment. Basil nanoemulsion at 60000ppm treated plots significantly reduced the insect number from 9.33±2.08 to 4.00±1 after 20 days of treatment. Turmeric oil nanoemulsion reduced the insect number, but the effectiveness was found to be lower than basil oil. The repeated measures of ANOVA results revealed a significant reduction in insect counts among the treatments and different time periods (Table 6).

Table 1. The characteristics of selected nanoemulsions of basil oil (B2) and turmeric oil (T4) were given as mean and standard deviation. ✓ - stable after centrifugation (7800 rpm/30 min)

Sample code	pH	EC (mS) (%)	Transmittance (d. nm)	Particle size (mV)	Zeta potential	PDI	Stability
B2	5.37± 0.72	0.10	37.03±47.02	97.33±52.13	-1.01±0.36	1.19±1.86	✓
T4	5.57± 0.12	0.10	91.60 ± 1.37	42.39± 10.76	-0.48±0.16	0.38±0.16	✓

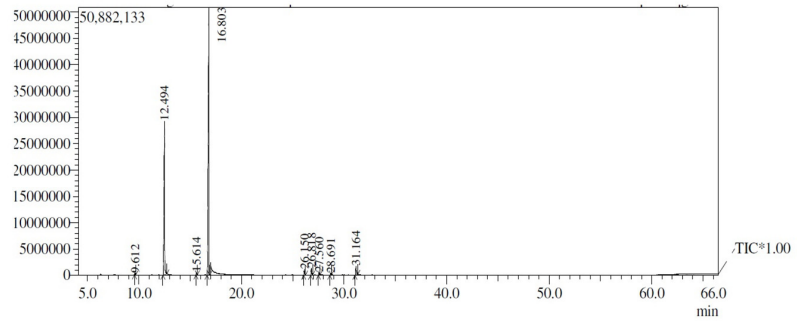


Fig.1

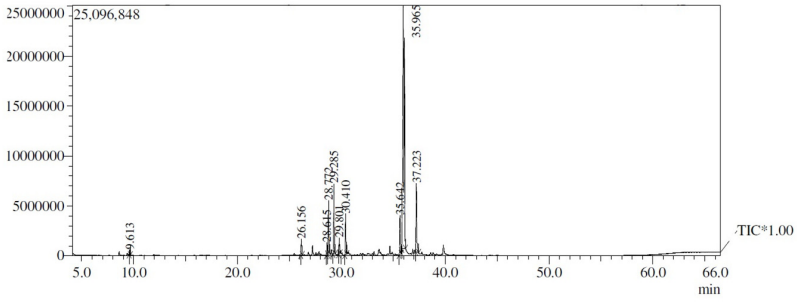


Fig.2.

Fig.1 Chromatogram of basil oil, Fig. 2 Chromatogram of turmeric oil

A mixture of volatile and semi-volatile organic compounds constitutes a plant essential oil. These compounds give the plants specific aroma, flavor, and fragrance (Moghaddam and Mehdizadeh, 2017). Basil is known for its various uses in various rituals, diseases, wound treatment, and as a deodorant. Phenylpropanoids and terpenes are the significant components of basil essential oil. However, the concentrations of these compounds vary in different geographic areas. The GC-MS analysis of basil oil has 55.92 per cent estragole (methyl chavicol) and 37.62 per cent linalool as the major components. Different chemotypes of basil were reported with different proportions of essential components by Ali and Setzerb (2013). Basil oil with high linalool (31.6%) content was reported from Pakisthan (Hussain *et al*., 2008), Bosnia and Herzegovina (Stanojevic *et al.,* 2017) and Egypt (Ahmed *et al.,* 2019). Basil oil with high Eugenol (70.3%) (Kim *et al.,* 2021), Linalool (Ling chang *et al.,* 2009) and methyl chavicol (88.6%) (Al-Harbi *et al.,* 2021) were also reported earlier. Turmeric essential is known for its antioxidant, anti-inflammatory, antidiabetic, anticancer, analgesic, cardiovascular, neuroprotective, nephroprotective, antibacterial, antifungal, antiparasitic, and insecticidal activities. Ar- Turmerone (63.45%) was the major component of commercially purchased turmeric essential oils. Previous studies of *Curcuma longa* samples from Kerala and Karnataka reported that have ar- turmerone as the major component (Gounder and Lingamallu, 2012; Liju *et al.,* 2013).

A stable nanoemulsion needs smaller-sized micelles and uniformity in their particles (Jaiswal *et al.,* 2015). Basil oil nanoemulsion B2 size range was 97.33±52.13 d.nm was in accordance with the previous records of 119±1.13 (da Silva Gündel *et al.,* 2018), 17.47 (Al-Okbi *et al.,* 2020) and 28nm (Ghosh *et al.,* 2014). In earlier studies of turmeric oil nanoemulsions, the size of micelles was 150.2

Table 2. Ovicidal effect of basil oil nanoemulsion (B) and turmeric oil nanoemulsion (T) on the hatching rate of *Leptocorisa oratoria* after 7 days of incubation

Treatment	Conc	Hatching rate (%)	Inhibition rate (%)	EII
Control	Control	100.00±0.00^{a}	0.00±0.00	0.000±0.000^{c}
B (ppm)	60000	0.00±0.00^{c}	100.00±0.00^{a}	1.000±0.000^{a}
	50000	5.33±1.15^{c}	94.67±1.15^{a}	0.947±0.012^{a}
	20000	34.67±5.03^{b}	65.33±5.03^{b}	0.653±0.050^{b}
	10000	39.33±4.16^{b}	60.67±4.16^{b}	0.607±0.042^{b}
	5000	92.66±3.05	7.33±3.05	0.0733±0.028^{c}
	F Value	*708.442*	*708.442*	*708.442*
	P Value	*0.000*	*0.000*	*0.000*
T	60000	0.00±0.00^{e}	100.00±0.00^{a}	1.000±0.000^{a}
	50000	10.00±2.00^{d}	90.00±2.00^{b}	0.900±0.020^{b}
	20000	22.67±2.31^{c}	77.33±2.31^{c}	0.773±0.023^{c}
	10000	37.33±1.15^{b}	62.67±1.15^{d}	0.627±0.012^{d}
	5000	89.00±2.00^{a}	11.33±2.30	0.110±0.020^{e}
	F Value	*2255.528*	*2255.528*	*2255.528*
	P Value	*0.000*	*0.000*	*0.000*
PC	Positive Control	0.00±0.00	100.00±0.00	1.000±0.000

Each value is the mean of three replicates. One way ANOVA used for comparing the values of mean values hatching rate, inhibition rate, and Effective Inhibition rate Index (EII) among the different concentrations of insecticides. The mean values having different superscripts are statistically significant ($P<0.05$), Tukey Post hoc test

Table 3. LC_{50} of basil oil nanoemulsion (B) and turmeric oil nanoemulsion (T) on eggs of *L. oratoria*

Treat	LC50 (ppm) (LL-UL)	Regression equation	R^2	χ^2 (DF)
B	13724 ppm (6834-19305)	y = 0.84 + 0.0000519x	0.730	60.73 (12)
T	14337 ppm (6699-21066)	y = 0.79 + 0.0000469x	0.646	111.50 (12)

Lower confidence limit (LL), upper confidence limit (UL), regression coefficient (R^2) and DF is degree of freedom

(Lin *et al.,* 2021) and 173.31 ± 2.24 nm (Shah *et al.,* 2024). In this study prepared turmeric nanoemulsion T4 of smallest-sized particles of 42.39± 10.76 d. nm. T4 seems to be the smallest-sized turmeric nanoemulsion reported in the previous studies. The uniformity of particles can be determined by polydispersity index (PDI) (Jaiswal *et al.* 2015). Basil and turmeric oil nanoemulsions showed PDI of 1.19±1.86 and 0.38±0.16. Higher uniformity of particles was observed in turmeric nanoemulsion than in basil oil nanoemulsion. Zeta potential between ±30 mV is needed for the physical stability of nanoemulsions (Gurpreeth and Singh, 2018). Both B2 and T4 have -1.01±0.36 and -0.48±0.16 zeta potential showing high physical stability. They were used for further assays in eggs and adults of *L. oratoria*.

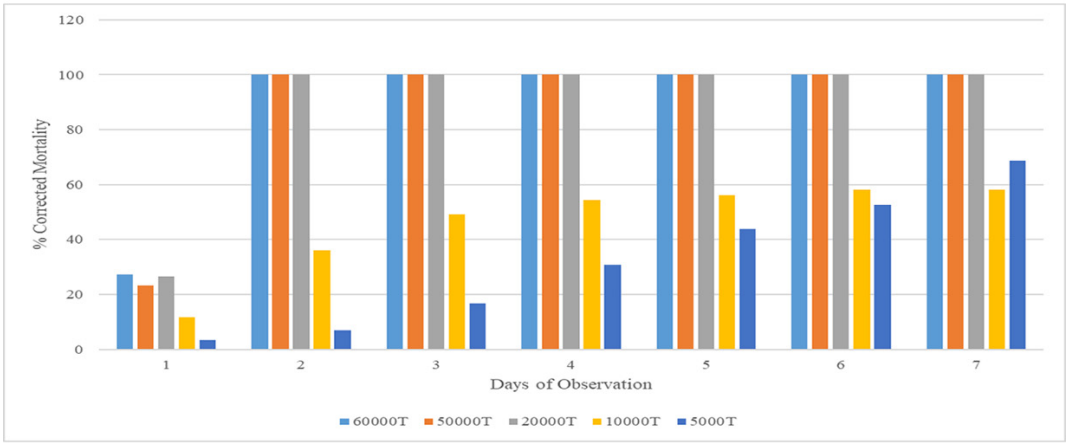
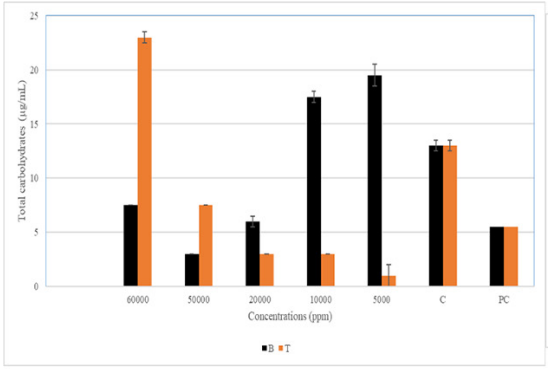


Fig.3.



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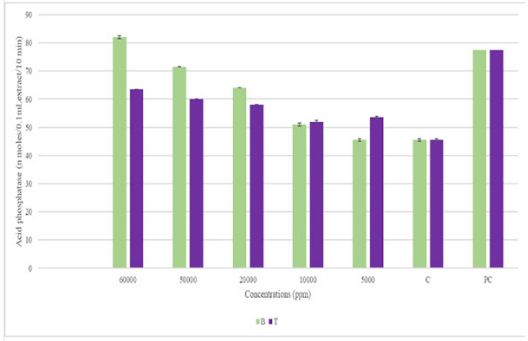

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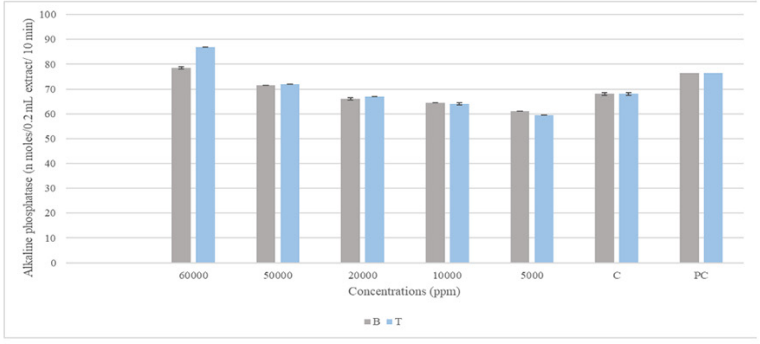

Fig.6.

Fig. 3 Corrected mortality per cent of adult *Leptocorisa oratoria* in turmeric oil nanoemulsions with different concentrations for 7 days

Fig. 4 Total carbohydrate values of *L. oratoria* in different concentrations of basil and turmeric nanoemulsions

Fig. 5 Acid Phosphatase activity in different concentrations of basil and turmeric nanoemulsions

Fig. 6 Alkaline phosphatase activity in different concentrations of basil and turmeric nanoemulsions

Table 4. Mortality of *Leptocorisa* 3, 5, 7, 10, 15 and 20 days after the treatments of nanoemulsions

Treatment	Conc ppm	3DAT	5DAT	7DAT	10DAT	15DAT	20DAT
Control		5.00±8.66^{d}	8.33±10.41^{c}	8.33±10.41^{c}	13.33±5.77^{c}	15.00±5.00^{c}	18.33±7.64c
B	60000	78.33±20.21^{b}	81.67±23.09^{b}	83.33±20.21^{b}	86.67±23.09^{b}	88.33±20.21^{b}	100.00±0.00^{a}
	50000	48.33±25.17^{c}	61.67±23.63^{b}	66.67±23.09^{b}	71.67±14.43^{b}	71.67±14.43^{b}	91.67±14.43^{b}
T	60000	81.67±2.89^{b}	85.00±5.00^{b}	88.33±7.64^{b}	90.00±10.00^{b}	91.67±7.64b	95.00±8.66b
	50000	70.00±18.03^{b}	73.33±17.56^{b}	73.33±17.56^{b}	80.00±8.66^{b}	80.00±8.66a	80.00±8.66^{b}
Positive Control	PC	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00a	100.00±0.00^{a}	100.00±0.00a

Each value is the mean of three replicates. The mean values having different superscripts are statistically significant (P<0.05), Tukey Post hoc test

Eggs treated with 60000 ppm of basil and turmeric oil nanoemulsions showed zero per cent hatching rate, indicating a 100 per cent inhibition rate and an EII as one. An effective inhibition rate index of one indicates that the formulation is as effective as the positive control used (malathion). The LC_{50} value of basil oil was found to be lower than turmeric oil, pointing to the high toxicity of basil oil. Basil oil is an excellent ovicidal agent for *Aedes aegypti* L. (Warikoo *et al.,* 2011) and *Musca domestica* (Senthoorraja *et al.,* 2021). A combination of ethanolic extract of turmeric and green tea showed a good ovicidal effect in *Trichuris* sp. (Hidayatik *et al.,* 2024). Besides these studies, basil and turmeric oil's ovicidal activities were not previously studied. This study is the first of this kind, and it revealed the efficacy of basil and turmeric oil in inhibiting the growth of eggs.

In the adulticidal assay, the trend of toxicity of basil and turmeric nanoemulsions varied greatly. Basil oil nanoemulsion showed maximum toxicity in 24 hours compared to turmeric nanoemulsion. Within 72 hours, complete mortality was observed in all concentrations of basil nanoemulsion treatments in lab conditions. The lethal dose by basil nanoemulsion was much lower than used for ovicidal activity. In the pot culture study, 60000ppm of basil nanoemulsion was found to be effective in controlling the. In the fields, the insect count was found to be reduced for basil oil-treated plots after 20 days. Basil oil and its constituents, such as estragole and linalool were found to have high insecticidal activity in *Ceratitis capitata, Bactrocera dorsalis* and *Bactrocera cucurbitae* (Ling Chang *et al.,* 2009). Estragole is a phenolic compound with a spicy aroma. *Sitophilus zeamais* and *Tribolium castaneum* showed contact toxicity to estragole (Huang *et al.,* 2001). Estragole can be easily degraded due to low chemical stability. It is also prone to oxidation and chemical interaction with other compounds. By encapsulating estragole, the solubility in water can be increased, thus increasing the absorption and reducing the degradation of estragole (Ulanowska and Olas, 2021). Linalool also showed contact toxicity to *S. zeamais* (Wang *et al.,* 2011) By preparing nanoemulsion of basil oil, the encapsulation increased the solubility of estragole and Linalool in water and thus increased the penetration and availability of estragole. Estragole and linalool can be the reason for the high mortality rate of basil nanoemulsion after 72 hours of lab treatment, 20 days of pot culture and in field study.

Insecticidal activities of turmeric essential oil on *Anopheles gambiae* (Kemabonta *et al.,* 2018), *Lucilia cuprina* (Chaaban *et al.* 2019), *Paederus fuscipus* and *Luprops tristis* (Vineesh *et al.,* 2022) were reported earlier. A*r*-turmerone caused 100 and 64 per cent mortality at 1,000 and 500 ppm respectively, on *Nilaparvata lugens* female adults and against *Plutella xylostella* larvae, the compound gave 100 and 82 per cent mortality at 1000 and 500ppm (Lee *et al.,* 2001). Ar- tumerone has high contact toxicity to *S. zeamais* and *S. frugiperda*. Compared to estragole, ar- turmerone has higher stability and can remain in the

Table 5. Field study - (number m^{-2})

Treatment	Pre count	DAT 5	DAT 10	DAT 15	DAT 20
60000 B	9.33±2.08^{a}	5.33±1.53^{bc}	7.67±3.21^{bc}	3.33±1.15^{cd}	4.00±1.00^{cd}
50000 B	12.00±1.00^{a}	10.33±2.08^{abc}	11.67±2.89^{b}	7.00±2.00^{bc}	7.00±2.65bc
60000 T	10.67±1.15^{a}	7.67±2.52^{b}	6.33±2.08^{bc}	4.67±1.53^{bcd}	9.33±1.53^{b}
50000 T	12.33±6.81^{a}	9.00±4.58^{ab}	11.33±3.51^{b}	10.00±1.00^{b}	11.00±2.00^{b}
C	11.67±2.08^{a}	15.00±2.00^{a}	20.67±2.08^{a}	24.67±4.51^{a}	30.33±1.53^{a}
PC	14.00±7.21^{a}	1.33±0.58c	1.00±1.00^{c}	0.00±0.00^{d}	0.33±0.58^{d}
F Value	0.410	10.052	19.292	47.074	117.035
P value	0.833	0.001	0.000	0.000	0.000

Each value is the mean of three replicates. The mean values having different superscripts are statistically significant ($P<0.05$), Tukey Post hoc test

environment for a longer duration (de Souza Tavares *et al.,* 2013). By preparing nanoemulsion, their persistence and penetration can be increased. This accounts for the 100 per cent mortality rate in turmeric nanoemulsions at 60000, 50000 and 20000ppm 48 hours after treatment. It is also evident in the pot culture study that the initial mortality rate by turmeric nanoemulsion at 60000 ppm was 81 per cent after 3 days of treatment, which is higher than that of basil nanoemulsion (78%).

The biochemical analysis revealed that basil and turmeric nanoemulsions induced insect high-stress responses. The carbohydrate value was found to decrease with increasing concentrations of basil nanoemulsion. In turmeric oil, carbohydrates were found to increase with increasing concentrations. According to Tanani *et al.* (2012), the total carbohydrate amount found to be different with different insecticides. Acid and alkaline phosphatase activity was higher in the concentrations of basil and turmeric nanoemulsions. When red palm weevil *Rhynchophorus ferrugineus* is treated with some insecticides, their acid and alkaline phosphatase activity is found to be increased (Hamadah, 2019). *Spodoptera exigua* larvae infected with nuclear polyhedrosis virus showed similar acid and alkaline phosphatase activity (Sujak *et al.*,1978).

Table 6. Field study- Results of repeated measures of ANOVA insects counts of different treatment exposed to different time periods

Effect	Value	F	Sig.	
Time	Pillai's Trace	0.702	5.313^{b}	0.018
	Wilks' Lambda	0.298	5.313^{b}	0.018
	Hotelling's Trace	2.361	5.313^{b}	0.018
	Roy's Largest Root	2.361	5.313^{b}	0.018
Time Treatment	Pillai's Trace	2.040	2.499	0.005
	Wilks' Lambda	0.016	3.865	0.000
	Hotelling's Trace	13.213	4.955	0.000
	Roy's Largest Root	10.157	24.378^{c}	0.000

This study concluded that basil and turmeric essential oils can be prepared into smaller nanoemulsions (97.33±52.13 and 42.39± 10.76 d. nm) with suitable surfactant. In the nanoemulsions, the oils will be trapped inside the surfactant as micelles and disperse in the water. This smaller size of micelles thus increases penetration of oils by increasing water solubility, improving their persistence, and increasing their availability for a long time. The study revealed high toxicity of basil and turmeric nanoemulsions to eggs (LC_{50} - 13724 and 14337ppm) and adults of *L. oratoria*. Basil oil nanoemulsion showed good toxicity to *L. oratoria* in both pot culture and field studies after 20 days of treatment. Biochemical analysis also revealed the variation in total carbohydrates, acid, and alkaline phosphatases. These previous reports and the

current study show that basil and turmeric nanoemulsions can act as suitable insecticides in mitigating *L. oratoria*.

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A new black fly species of subgenus *Gomphostilbia* Enderlein, 1921 (Diptera, Simuliidae) from Eastern Ghats, India

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ABSTRACT: *Simulium* (*Gomphostilbia*) *alagarense* **sp. nov.,** was described based on adults (female and male), pupae, and larvae. This new species is placed in the *S. batoense* species group of the subgenus *Gomphostilbia* Enderlein by having an antenna with nine flagellomeres, pleural membrane bare, female subcostal bare, pupal gill composed of 8 slender threat-like filaments in the pupa, hypostome bristles per side lying parallel to lateral margin in the larvae. It is morphologically similar to *S.* (*G.*) *kumbakkaraiense* recently described in Tamil Nadu, south India (Anbalagan *et al*., 2019).
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KEY WORDS: Lotic dipterans, Karanthamalai, *S. batoense*

INTRODUCTION

Black flies (Family: Simuliidae) are significant both medically and veterinary-wise. Black flies spread many diseases, but the most common is onchocerciasis, or river blindness, by the parasitic worm *Onchocerca volvulus* (WHO, 2022; Gyasi *et al*., 2023). Blindness, skin sores, and excruciating itching can all result from this illness. Black flies can cause distress to livestock through their persistent biting, leading to reduced feeding and weight gain, lower milk production, and overall stress. Leucocytozoonosis is a disease caused by protozoan parasites of the genus *Leucocytozoon* (Piratae *et al*., 2021). These parasites infect birds and are transmitted by black flies (Simuliidae) and biting midges (Ceratopogonidae). Globally, 2415 black fly species were recorded (Adler, 2025), and 87 species (74 named and 13 unnamed) were found in India (Vijayan and Anbalagan, 2023). Among these 87 species belong to six subgenera in the genus *Simulium* Latreille (Adler, 2024): *Eusimulium* (one species named and one unnamed), *Gomphostilbia* (20 named and 2 unnamed species), *Montisimulium* (four named and one unnamed species), *Nevermannia* (eight named and two unnamed species), *Simulium* (40 named and seven unnamed species) and *Wilhelmia* (one species). Twenty-two species in *Gomphostilbia* in India were recorded under three species groups: *S.* (*G.*) *batoense* (13 species), *S.* (*G.*) *ceylonicum* (one species), *S.* (*G.*) *darjeelingense* (one species), *S.* (*G.*) *epistum*

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(three species), *S.* (*G.*) *gombakense* (two species) and *S.* (*G.*) *gomphostilbia* (two unplaced species). These species were assigned to the *S. batoense* species group of the subgenus *Gomphostilbia.* This study explores black flies in the Alagar hills of Eastern Ghats, south India. Among these specimens, a novel species of the genus *Gomphostilbia* Enderlein was found. Therefore, a description of this new species was provided.

MATERIALS AND METHODS

Larvae and pupae were collected manually with a fine brush and forceps from stream substrates (leaf litter, boulders, pebbles, and submerged human waste materials like polythene, snack covers, and cloths). Sterilized water was used for rearing to prevent introducing fungal spores into the system. Collected larvae and those not used for rearing pupae were preserved separately in the field in 70 per cent ethanol. The pupae were separated and placed on wet filter paper in a small plastic container for rearing. They were kept in an incubator adjusted to be similar to their habitat (21–27°C) for one or two days until adult emergence. Maintained regular inspection and adequate ventilation in the rearing area to prevent stagnant air and replaced rearing substrates to prevent the accumulation of organic matter that can promote fungal growth. Employed disinfectants, including 70 per cent ethanol, to sterilize all containers, instruments, and surfaces beforehand. This practice is effective in reducing microbial load and preventing fungal outbreaks (Hunter *et al.,* 1994; Jonusaite and Buda, 2002; Anbalagan *et al.,* 2011; Vijayan *et al*., 2017; Vasiliev and Aibulatov, 2023). Morphological characteristics of larvae, pupae, and adults were examined under a compound and stereo microscope. Dissected parts (heads, thoraxes, abdomens, legs, and wings) were cleared with lactic acid (85%) and transferred to glycerin for mounting. Permanent slides were made using a small drop of Canada balsam and were kept for two weeks without disturbing them to become dry. Clear nail polish was applied to the edges of the dried slides to be sealed. The mounting slides were examined under a Leica® DM300 LED compound microscope. Morphology characters were measured using a micrometer. Morphology characters were manually illustrated using a camera lucida attached to a Leica® MZ16 stereo microscope. Description, illustrations, and terms for morphological features were according to Takaoka (2003), Adler *et al.* (2004), Vijayan *et al.* (2019) and Anbalagan *et al.* (2020a). Dichotomous keys (Takaoka, 2012; Takaoka *et al*., 2019; Pramual *et al.,* 2022) were used for the identification of specimens. Emerging specimens were stored in ethanol at "20°C, and all type specimens were deposited at the Department of Animal Health and Management, Science Campus, Alagappa University, Karaikudi, Tamil Nadu, India (DAHMA).

RESULTS

Simulium (*Gomphostilbia*) *alagarense* Vijayan & Anbalagan sp. nov. (Figs. 1–3)

LSIDurn:lsid:zoobank.org:act:EAFBC97D-5FAA-4012-A8A1-D5606134789E

Diagnosis. Female adult: the only species of the *S. batoense* species group with antenna nine flagellomeres, pleural membrane bare, subcosta bare, and tibia light to dark brown with basal 1/4 white, hind basitarsus narrow and nearly parallel-sided, 6.1 times as long as wide, and 0.58 and 0.63 times as wide as greatest width of tibia and femur.

Female (n = 1). Body length 2.5–2.6 mm. ***Head***. Nearly as wide as the thorax. Frons grey, shiny, moderately covered with yellowish-white scale-like recumbent short hairs; frontal ratio 1.9:1.0:2.9; head ratio 1.0:5.9. Fronto-ocular area well developed, narrow, directed dorsolaterally. Clypeus brownish-black, densely covered with yellowish-white recumbent hairs interspersed with a few to several dark longer hairs on each side. Labrum 0.7 times as long as clypeus. Antenna composed of scape, pedicel, and nine flagellomeres, grey to white except scape, pedicle, and base of a first flagellomere dark yellow, proportional length of first and second flagellomeres 1.0:0.7. Maxillary palp composed of five palpomeres, dark brown, proportional length of third, fourth, and fifth palpomeres 1.0:1.1:2.3; third palpomere of moderate

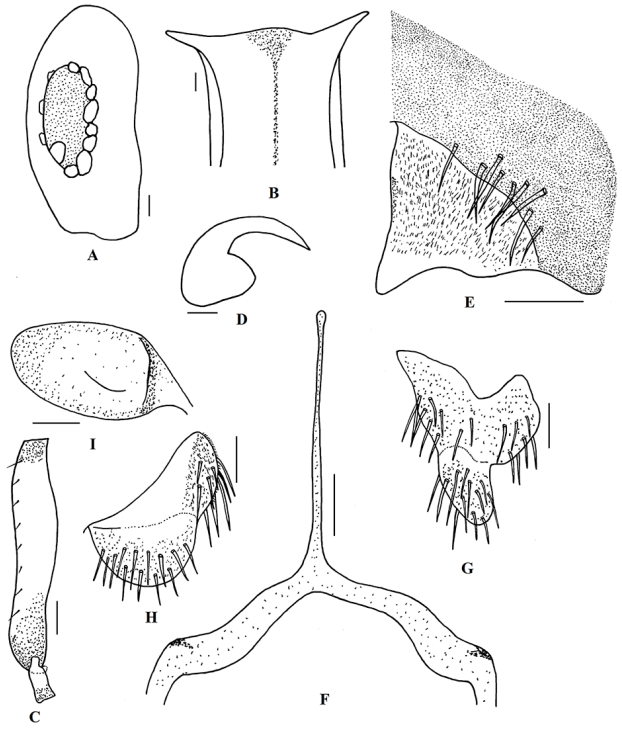

Fig. 1 Female of *Simulium* (*Gomphostilbia*) *alagarense* sp. nov. A, 3rd segment of right maxillary palp showing sensory vesicle (frontal view); B, cibarium (frontal view); C, basitarsus and 2nd tarsomere of left hind leg showing calcipala and pedisulcus (frontal view); D, tarsal claw; E, 8th sternite, ovipositor valve (ventral view); F, genital fork (ventral view); G & H, right paraprocts and cercus (G, ventral view; H, lateral view); I, spermatheca (lateral view). Scale bars. 0.01mm for B; 0.02mm for A, E and F; 0.05mm for G–I; 0.1mm for C

size (Fig. 1A); sensory vesicle ellipsoidal, long, 0.6 times length of third palpomereand with amedium-sized opening. Maxillary lacinia with 11 inner and 15 outer teeth. Mandible with 35 inner and 11 outer teeth. Cibarium (Fig. 1B) medially forming sclerotized plate folded forward from the posterior margin, with a strongly sclerotized medial longitudinal ridge with a well-sclerotized cup-like apex. ***Thorax***. Scutum dark brown except anterolateral calli dark ochreous, with 3 brownish-black longitudinal vittae (1 narrow median and 2 lateral), median vitta united anteriorly to anterior calli, lateral vittae united posteriorly to prescutellar area; scutum shiny when illuminated at certain angles, moderately covered with yellow scale-like recumbent short hairs interspersed with dark brown long upright hairs on prescutellar area. The scutellum is shiny, with dark brown short hairs. Postnotum brownish-yellow and bare. Pleural membrane bare. Katepisternum brownish-black to black, longer than deep, shiny when illuminated at certain angles, moderately covered with fine short hairs. ***Legs***. Foreleg: coxa yellow; trochanter yellow except apical portion somewhat darkened; femur light brown; tibia light brown; tarsus brownish black, with moderate dorsal hair crest; basitarsus slightly dilated, 5.3 times as long as its greatest width. Midleg: coxa yellowish brown except posterior surface dark brown; trochanter yellow to light brown; femur and tibia brown; tarsus brownish-black except basal 1/2 of basitarsus dark yellow. Hind leg: coxa yellowish brown; trochanter yellow; femur medium brown with base yellow and apical cap dark brown; tibia light to dark brown with basal 1/4 white, covered with brownish fine hairs on outer and posterior surfaces; tarsus brownish-black except basal 2/3 of basitarsus (though base light brown); hind basitarsus (Fig. 1C) narrow, nearly parallel-sided, 6.1 times as long as wide, and 0.58 and 0.63 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 1C) slightly shorter than width at base, and 0.57 times as wide as greatest width of basitarsus. Pedisulcus (Fig. 1C) well defined. Claw (Fig. 1D) with large basal tooth 0.4 times as long as claw. ***Wing***. Length 1.9 mm. Costa with dark brown spinules and light brown hairs. Hair tuft on a base of radial vein dark brown. The basal portion of the radius was fully haired. Basal cells absent. Halter. Clear white except for the margin and basal stem darkened. ***Abdomen***. Basal scale dark yellow, with a fringe of yellowish-white hairs. Dorsal surface of the abdomen is medium to dark brown except for segment 2 light brown though middle portion of the tergal plate is ochreous, and moderately covered with dark short to long hairs; tergites of segments 2 and 6–9 is shiny when illuminated at certain angles. The ventral surface of segments 2–4 is creamy, and those of other segments are light to dark brown; sternal plate on segment 7 is undeveloped. ***Genitalia***. Sternite 8 (Fig. 1E) is well sclerotized and bare medially, covered with 7–9 long hairs on each side. Ovipositor valves are tongue-like, thin, membranous, and moderately covered with

microsetae; inner margins are convex, somewhat sclerotized, and slightly separated from each other. Genital fork (Fig. 1F) of usual inverted-Y forms; stem slender and well sclerotized; arms of moderate width, lateral plate of each arm with thin lobe directed posteromedially and small stout projection directed anterodorsally. Paraproct in ventral view concave anterolaterally, with 3 sensilla on an anteromedial surface; paraproct in lateral view (Fig. 1G) much produced ventrally, 0.54 times as long as wide, with 3–5 medium-long too long hairs on ventral and lateral surfaces. Cercus in lateral view (Fig. 1H) is short, rounded posteriorly, 0.55 times as long as wide. Spermatheca (Fig. 1I) ellipsoidal, 1.8 times as long as its greatest width, well sclerotized except duct and a small area near the juncture with duct unsclerotized, and with a hexagonal pattern on the surface; internal setae absent; both accessory ducts slender, subequal in diameter to major one.

Fig. 2 Male of *Simulium* (*Gomphostilbia*) *alagarense* **sp. nov.** A, 3rd segment of left maxillary palp showing small sensory vesicle (frontal view); B, basitarsus and 2nd tarsomere of left hind leg showing calcipala and pedisulcus (frontal view); C, coxites, styles, ventral plate and median sclerite (ventral view); D, left style (frontal view); E, ventral plate and median sclerite (lateral view); F, ventral plate (end view); G, left paramere and aedeagal membrane (hind view); H, 10th abdominal segment and cercus (right side and lateral view). Scale bars. 0.02mm for A, G and H; 0.05mm for C–F; 0.1mm for B

Male (n = 1). Body length 3.2 mm. ***Head***, somewhat wider than thorax. Upper eye yellowish brown, consisting of13 vertical columns and 13 horizontal rows of large facets. Face brownish black, grayish-white pruinose. Clypeus brownish black, whitish pruinose, densely covered with golden-yellow scale-like medium-long hairs (directed upward and lateral) interspersed with several dark brown simple longer hairs. Antenna 1st flagellomere elongates, 1.7 times as long as 2nd one. Maxillary palp light to medium brown, proportional lengths of 3rd, 4th, and 5th segments 1.0:1.1:2.1; 3rd segment (Fig. 2A) widened apically; sensory vesicle (Fig. 2A) ellipsoidal, small (0.25 times as long as 3rd segment), and with medium-sized opening. ***Thorax***. Scutum is slightly darker than female and short hairs on the scutum is golden yellow. ***Legs***. Foreleg: coxa yellow; trochanter yellow with some portions light brown; femur light brown except apical cap brown; tibia brown with median 2/3 light brown and covered with dark brown hairs; tarsus brown to dark brown; basitarsus moderately dilated 6.6 times as long as its greatest width. Midleg: coxa yellowish brown; trochanter yellow to brown; femur yellow except apical 1/4 brown; tibia medium brown to dark brown; tarsus dark brown to brownish-black except the anterior surface of little less than basal 1/2 of basitarsus dark yellow to light brown. Hindleg: coxa dark yellow to brown; trochanter yellow; femur light brown except apical 1/2 dark brown; tibia brown except basal and apical portion dark brown; tarsus medium to dark brown except basal 1/2 (or little less) of basitarsus whitish-yellow and little less than basal 1/3 of 2nd tarsomere white; basitarsus (Fig. 2B) slender, spindle-shaped, 4.7 times as long as wide, and 0.6 and 0.7 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 2B) nearly as long as wide, and 0.39 times as wide as greatest width of basitarsus. Pedisulcus (Fig. 2B) well defined. ***Wing***. Length 1.9 mm. Costa with dark brown spinules as well as dark brown hairs

except the basal portion with a patch of yellowish hairs. Subcosta bare. Hair tuft on stem vein dark brown. Basal portion of radius fully haired; $R^1$with dark spinules and hairs and R^2 with hairs only. Basal cells absent. Halter. Yellowish brown except outer surface ochreous, basal stem darkened, and apex white. ***Abdomen***. Basal scale dark brown, with a fringe of light to medium brown hairs. Dorsal surface of abdomen is dark brown except for segment 2 light brown (though posterior 1/4 of dorsal surface brown), covered with dark brown short to long hairs; segments 2, 5–7 with shiny dorsolateral or lateral patches; ventral surface of segment 2 yellow, those of segments 3 and 4 yellow except sternites medium brown, and those of other segments medium to dark brown. ***Terminalia***.Coxite in ventral view (Fig. 2C) is nearly rectangular, 1.7 times as long as its greatest width. Gonostyle in ventral view slightly bent inward, slightly tapered from base to apex, sharped apically and with apical spine; style in medial view (Fig. 2D) longer than coxite (0.71 times as long as coxite), gently bent inward, nearly parallel-sided, with apical spine; style in ventrolateral view very slightly tapered toward apical 3/4, with rounded apex. Ventral plate in ventral view (Fig. 2C) with body transverse, 0.81 times as long as wide, tapered posteriorly, with anterior margin produced antero-medially, and posterior margin concave medially, densely covered with microsetae on ventral surface; basal arms of long, directed forward, then convergent apically; ventral plate in lateral view (Fig. 2E) moderately produced ventrally; ventral plate in end view (Fig. 2F) triangular dorsally, without microsetae on posterior surface. Median sclerite is thin, plate-like, wide. Paramere (Fig. 2G) of moderate size, with 3 distinct long and stout hooks and several smaller ones. Aedeagal membrane is moderately setose and slightly sclerotized at the base but the dorsal plate is not well defined. Ventral surface of abdominal segment 10 without distinct hairs near posterior margin. Cercus in lateral view (Fig. 2H) is small, and rounded, with 8–10 hairs.

Pupa (n = 4). Body length 3.1–3.2 mm. ***Head***. Integument dark yellow, moderately covered with small round tubercles; antennal sheath with protuberance; face with pair of simple very long

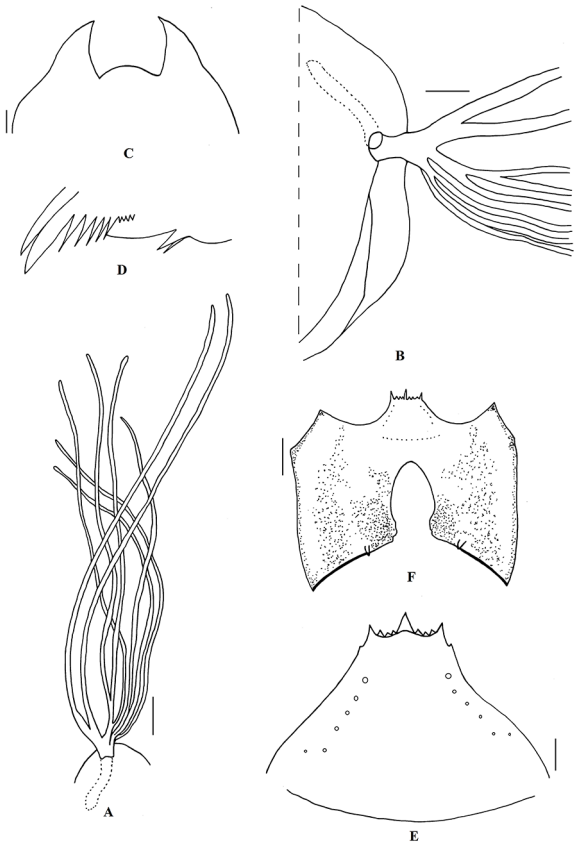

Fig. 3 Pupa and larva of *Simulium* (*Gomphostilbia*) *alagarense* **sp. nov.** A–C, pupa; D–F, larva. A, left gill filaments (lateral view); B, basal portion gill (outer view); C, terminal hooks (hind view); D, right mandible (lateral view); E, hypostoma (ventral view); F, head capsule showing postgenal cleft and hypostoma (ventral view). Scale bars. 0.01mm for D; 0.02mm for E; 0.05mm for C; 0.1mm for A, B and F

trichomes with coiled apices, and frons with 3 pairs of simple very long trichomes with coiled apices; 2 frontal trichomes on each side arising close together, subequal in length to one another and slightly longer than facial one. ***Thorax***. Integument yellow, covered with round tubercles, with 3 simples very long dorsomedial trichomes with coiled apices, 1 simple very long anterolateral trichome with coiled apices, 1 simple very long mediolateral trichome with uncoiled apex, and 3 simples ventrolateral trichome with uncoiled apices (1 long and 2 short) on each side. Gill (Fig. 3A) composed of 8 slender thread-like filaments, arranged as [(1 + 2) + (1 + 2)] + 2 filaments from dorsal to ventral, with medium-long common basal stalk having somewhat

swollen transparent organ ventrally at base (Fig. 3B); common basal stalk 0.5 times length of interspiracular trunk; both primary and secondary stalks of dorsal triplet short, primary stalk of middle triplet medium-long, but secondary stalk short; length of primary and secondary stalks of middle triplet combined slightly longer than stalk of ventral pair; stalk of ventral pair short, 0.9–1.0 times length of common basal stalk and 0.55–0.6 times length of interspiracular trunk; stalk of ventral pair 1.5–1.52 times as thick as primary stalk of middle triplet, and 1.7–1.8 times as thick as primary stalk of dorsal triplet; primary stalk of dorsal triplet lying against stalk of ventral pair at angle of 40–60 degrees or little more when viewed laterally; all filaments yellowish brown, gradually tapered toward apex; entire length of filaments (measured from base of gill to tips of filaments) based on one pupa as follows: 1.5–1.6 mm for dorsal triplet, 2.0–2.1 mm for middle triplet and 2.7–2.8 mm for ventral paired filaments. Abdomen. Segment 9 with a pair of triangular flat terminal hooks, of which the outer margin is slightly longer than the inner margin and not crenulated (Fig. 3C). ***Cocoon*** (n = 8). 3.3–3.5 mm long by 3.0–3.1 mm wide; wall pocket-shaped, thinly and moderately woven, widely extended ventrolaterally; anterior margin somewhat thickly woven, with the dorsal portion not or slightly produced anteriorly when viewed dorsally; posterior 1/2 with floor roughly or moderately woven.

Mature larva (n = 8). Body length 5.8–5.9 mm. Body creamy to colour markings as follows: thoracic segment 1 encircled with an ochreous broad transverse band (though disconnected ventrally), proleg greyish, thoracic segments 2 and 3 yellowish-grey dorsally and each with distinct ochreous wide areas ventrally, abdominal segments 1–4 each encircled with yellowish brown broadband, abdominal segments 5–8 almost entirely covered by yellowish brown transverse broadband on dorsal and dorsolateral surfaces; abdominal segments 5 and 6 each with W-shaped broad transverse greyish-brown band on dorsolateral surfaces of posterior 1/2 of each segment; abdominal segment 7 and 8 with a transverse yellowish-brown band on ventral surface; Cephalic apotome yellowish brown, and sparsely covered with simple minute setae; head spots indistinct. Lateral surface of the head capsule is yellowish brown except the eye-spot region yellow, and very sparsely covered with simple minute setae; spots indistinct. Ventral surface of the head capsule is yellowish brown except a somewhat darkened area near the posterior margin on each side of postgenal cleft, and very sparsely covered with simple minute setae. Antenna composed of 3 segments and apical sensillum, 1.4 times longer than the stem of labral fan; proportional lengths of 1st, 2nd, and 3rd segments 1.00:1.2:1.4. Labral fan with 26–28 main rays. Mandible (Fig. 3D) with 3 comb-teeth decreasing in length from 1st to 3rd; mandibular serrations composed of 2 teeth (1 medium-sized and 1 small); major tooth at an acute angle against mandible on the apical side; supernumerary serrations absent. Hypostoma (Fig. 3E) with a row of 9 apical teeth; median and each corner tooth prominent (though median tooth slightly longer than corner teeth) and much longer than 3 intermediate teeth on each side; lateral margin smooth; 5 hypostomal bristles per side lying parallel to the lateral margin. Postgenal cleft (Fig. 3F) is arrowhead-shaped, 2.7 times as long as postgenal bridge. Cervical sclerite is composed of 2 very pale small pieces, not fused to occiput, moderately separated medially from each other. Anal sclerite of usual X-form, with anterior arms a little shorter (0.71 times as long as posterior arms) than posterior ones, broadly sclerotized at base; accessory sclerite absent. Posterior circlet with 68–70 rows of hooklets with up to 13–14 hooklets per row.

Type series. Holotype (in alcohol): Female (with associated pupal exuviae and cocoon, in 80% ethanol), collected from a small stream (width 1–1.5 m, depth 9cm, water temperature 23.5°C, more than partially shaded, elevation 363m, 10°17'23.51'' N; 78°14'44.38'' E), slow running in a natural forest, Ayyan odai, Malaiyur, Karanthamalai, Dindigul district, Tamil Nadu, India, 16-XII-2021, by S. Vijayan & S. Anbalagan, Paratypes: (in alcohol) One female, one male, eight pupae, thirteen mature larvae and twenty-one immature larvae, same data as those of the holotype.

Biological notes. Able to collect the larvae and pupae of this newly discovered species off the leaves. Associated simuliids were *S*. (*S*.) *striatum*,

and *S.* (*G.*) **sp. n1.**

Distribution. Ayyan odai, Ayyan aruvi, Uluppakkudi Falls, Nuburagangai, Periaythoppu stream, Sithan aruvi, and Mavoor stream.

Etymology. The species name *alagarense* refers to the name of Alagar Hill, where this new species was collected.

Remarks. The following morphological characteristics of *Simulium* (*G.*) *alagarense* sp. nov. place it in the subgenus *Gomphostilbia* in *Simulium*: haired katepisternum in the adults, abdominal segment 9 with pair of triangular flat terminal hooks in the pupa and smooth lateral margins of hypostoma in the larva. In addition, this new species is assigned to the *Simulium batoense* species group of the subgenus *Gomphostilbia* by having a bare pleural membrane, dark brown hair tuft on the base of the radius, dark tibiae of the female and male, slender male hind basitarsus, eight-gill filaments, and grapnel-like hooklets in the pupa (Takaoka, 2012).

Simulium (*G.*) *alagarense* sp. nov., is morphologically similar to *S.*(*G.*) *kumbakkaraiense* recently described from Tamil Nadu, South India (Anbalagan *et al*., 2019) in sharing the following characteristics: labrum 0.7 times as long as clypeus, scutum with three brownish-black longitudinal vittae and tarsal claw with large basal tooth in the female, body length of male 3.2 mm, the respiratory gill with 8 slender thread-like filaments in the pupa and wall pocket-shaped cocoon and antenna proportional length of 1st, 2nd and 3rd segments 1.0: 1.2: 1.2 in the larva.

However, this new species is distinguished from *S.*(*G.*) *kumbakkaraiense* by the following characters (those of *S.* (*G.*) *kumbakkaraiense*s in parentheses):in the female by the hind basitarsus 6.1 times as long as wide (5.3 times as long as wide) and sternite 8 covered with 9 long hairs on each side (10–13 long hairs on each side), in the male by the antenna 1st flagellomere elongates, 1.3 times as long as 2nd one (antenna 1st flagellomere elongates, 1.7 times as long as 2nd one) and fore basitarsus 6.6 times as long as its greatest width (6.0 times as long as its greatest width), in the pupa by the common basal stalk 0.41 times length of interspiracular trunk (1.0 times length of interspiracular trunk) and in the larva by the postgenal cleft 2.7 as long as postgenal bridge (1.5 as long as postgenal bridge).

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Microbial analysis of hive stored pollen of Indian honey bee, *Apis cerana indica* (Fabricius) (Hymenoptera, Apidae)

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ABSTRACT: The study was conducted to identify the microbial communities associated with the hive stored pollen of *Apis cerana indica* colonies in Palakkad and Wayanad region of Kerala. Investigation revealed that samples collected from Palakkad contained two bacteria *viz., Pseudomonas aeruginosa* and *Bacillus megaterium* and one fungus *viz*., *Trichoderma reesei,* while pollen from Wayanad had two bacteria *viz*., *B. aryabhattai* and *B. megaterium* and two fungi *viz., Westerdykella multispora* and *Neurospora crassa*. Microbial load was more in samples from Wayanad ($6.6x10^4$ cfu g^{-1}) compared to Palakkad sample ($3.3x10^4$ cfu g^{-1}). © 2025 Association for Advancement of Entomology

KEY WORDS: Microbial communities, bacteria, fungi, microbial load

INTRODUCTION

Honey bee colonies rely heavily on flower nectar and pollen for nutrition, which requires protection from microbial degradation. Bee substances like plant resins, nectar, pollen, wax, honey, and propolis exhibit antimicrobial properties, playing a crucial role in preserving stored pollen (Kujumgiev *et al.,* 1999; Ohashi *et al.,* 1999). Microbial activity (microbial succession, anaerobic breakdown and pollen predigestion) improves the nutrition of stored bee bread, which has been reported to possess antimicrobial, antioxidant, and anti-radiation properties (Loper *et al.,* 1980; Anderson *et al*., 2011; Matilla *et al.,* 2012; Homan *et al*., 2013; Disayathanoowat *et al.,* 2020). There are diverse and abundant microbial communities in the bee pollen that not only play roles in pollen processing and preservation (Martinson *et al.,* 2011; Corby-Harris *et al.,* 2014), but also may serve as significant protein sources for developing bees. The microbial composition in pollen and hive-stored bee bread is influenced by various factors, including land use, floral sources and seasonal variation (Anderson *et al*., 2014; De Palma *et al.,* 2016; Anderson *et al*., 2013). While the chemical composition of bee pollen from different plants and geographical origins has been well-studied, information on microbial diversity in hive-stored pollen is limited. This knowledge gap is significant, particularly considering the importance of bee pollen in human nutrition and the potential risks associated with microbial contamination. Studies have shown that diverse microbial communities are associated with honey bee gastrointestinal tracts and hive-stored pollen, highlighting the need for further research. This study

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aims to investigate the microbes associated with hive-stored pollen of *Apis cerana indica* colonies, contributing to our understanding of pollen preservation and informing microbiological standards for safe consumption.

MATERIAL AND METHODS

The study conducted at Department of Agricultural Entomology, College of Agriculture, Vellanikkara, Kerala Agricultural University during 2020-2021. Survey was conducted for the collection of hive stored pollen from the bee hives of Indian honeybees. Samples were collected from two different locations *viz.,* Palakkad and Wayanad districts, which represented the central midlands and highland areas of Kerala. The samples collected from Palakkad were given the code PKD, which includes PKD-1: Mannarkkad, PKD-2: Nellipuzha, PKD-3: Nottanamala and similarly, from Wayanad as WYD, which includes WYD-1: Kenichira, WYD-2: Kayakunnu and WYD-3: Cheengode. Serial dilution and plate counting technique was followed for isolation of microbes and readymade media was used such as nutrient agar (MOO1), Potato Dextrose Agar (MHO96), Kenknight and Munaier's medium (M695) and Sabouraud Dextrose Agar, granulated medium (GM063) were used to isolate bacteria, fungi, actinomycetes and yeast, respectively. The media were prepared and autoclaved for 20-30 minutes at 121°C and 15 lbs of pressure. The medium was stored at room temperature for further use. One gram of Indian bee pollen samples was serially diluted up to 10^{-4} and a series of dilutions were performed. The samples collected from each location were plated carefully in respective media for the isolation of bacteria, fungi, actinomycetes and yeast using nutrient Agar (NA), Potato Dextrose Agar (PDA), Kenknight and Munaier's medium and Sabouraud Dextrose Agar (SDA), respectively. Three replications were maintained for each sample. After incubation, colony-forming units were calculated (Anderson *et al.,* 2014). Bacterial colonies were sub-cultured by performing quadrant streaking method on NA media and purified by performing repeated streaking on NA slants. Fungal colonies were sub-cultured by placing the hyphal disc at the centre of PDA media and purified by placing the hyphal discs in PDA slants. Morphological characters of bacteria were identified by performing gram staining and fungi isolates were identified by slide preparation and under phase-contrast microscope. Further, molecular characterization of bacterial isolates was carried out based on 16S rRNA gene sequencing.

Sequencing of ITS region of fungi and bacteria: The 16S rRNA and ITS (The Internal Transcribed Spacer) regions of bacteria and fungi were amplified with universal primers specific to bacteria and fungi. Purified cultures of bacteria and fungi were given for sequencing at the Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala. Genomic DNA of bacteria was isolated using the NucleoSpin® Tissue Kit (Macherey-Nagel) following the manufacturer's instructions. Part of the culture was taken in a microcentrifuge tube. At 56°C, 180 ìl of T1 buffer and 25 ìl of proteinase K were added and incubated in a water bath until they were completely lysed. After lysis, 5 ìl of RNase A (100 mg ml^{-1}) was added and incubated at room temperature for 5 minutes. After incubating at 70°C for 10 minutes, 200 ml of B3 buffer was added and incubated for another 10 minutes. Then 210 ìl of ethanol (100%) was added and mixed thoroughly by vortexing. The mixture was pipetted into a NucleoSpin® Tissue column placed in a 2ml collection tube and centrifuged at 11000 x g for 1 minute. The NucleoSpin® Tissue column was transferred to a new 2ml tube and washed with 500ìl of BW buffer. The wash step was repeated using 600ìl of B5 buffer. After washing, the NucleoSpin® Tissue column was placed in a clean 1.5ml tube and DNA was eluted out using 50ìl of BE buffer. The quality of the DNA isolated was checked using agarose gel electrophoresis. One ìl of 6X gel-loading buffer (0.25% bromophenol blue, 30% sucrose in TE buffer pH-8.0) was added to 5ìl of DNA. The samples were loaded onto a agarose gel (0.8%) prepared in 0.5X TBE (Tris-Borate-EDTA) buffer containing 0.5 ìg ml^{-1} ethidium bromide. Electrophoresis was performed with 0.5X TBE as electrophoresis buffer at 75 V until the bromophenol dye front had migrated to the bottom of the gel.

The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using the Gel Documentation System (Bio-Rad). The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) by using universal primers. Five ìl of PCR product was mixed with 0.5ìl of ExoSAP-IT and incubated at 37°C for 15 minutes followed by enzyme inactivation at 85°C for 5 minutes.

For fungus, about 100mg of the fungal tissue or mycelium was homogenized using liquid nitrogen and the powdered tissue was transferred to a microcentrifuge tube. Four hundred microlitres of buffer PL1 was added and vortexed for one minute. 10 µl of RNase A solution was added and inverted to mix. For 10 min, the homogenate was incubated at 65!.The lysate was transferred to a Nucleospin filter and centrifuged at 11000 x g for 2 min. The flow through liquid was collected and the filter was discarded. 450 µl of buffer PC were added and mixed well. The solution was transferred to a Nucleospin Plant II column, centrifuged for 1 min and the flow through liquid was discarded. The column was filled with 400 µl of buffer PW1, centrifuged at 11000 x g for 1 min and the flows through liquid was discarded. Then 700ìl PW2 was added, centrifuged at 11000 x g and the flow through liquid was discarded. Finally, 200ìl of PW2 was added and centrifuged at 11000 x g for 2 minutes to dry the silica membrane. The column was transferred to a new 1.7 ml tube and 50 ìl of buffer PE was added and incubated at 65°C for 5 minutes. The column was then centrifuged at 11000 x g for 1 minute to elute the DNA. The eluted DNA was stored at 4°C. Gel electrophoresis was carried out in the same way for bacterial DNA. The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) by using universal primers.

Agarose Gel electrophoresis of PCR products*:* The PCR products were checked on 1.2 per cent agarose gels prepared in 0.5X TBE buffer containing 0.5ìg ml^{-1} ethidium bromide. For loading, 1ìl of 6X loading dye was mixed with 4ìl of PCR products and electrophoresis was performed at 75V power supply with 0.5X TBE as electrophoresis buffer for about 1-2 hours, until the bromophenol blue front had migrated to almost the bottom of the gel. The molecular standard used was a 2-log DNA ladder (NEB). The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using the Gel Documentation System (Bio-Rad).

Exo SAP-IT Treatment*:* Exo SAP-IT (USB) consists of two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP), in a specially formulated buffer for the removal of unwanted primers and dNTPs from a PCR product mixture with no interference in downstream applications.

NCBI Blast*:* The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1. The rRNA homology searches were performed using the BLAST program.

The pollen sample data analysis with three replications was carried out. Using R software, the obtained data were analysed using Welchs's two sample t test.

RESULTS AND DISCUSSION

In the investigation, two bacteria and one fungus were identified from Palakkad sample, whereas from Wayanad sample two bacteria and two fungi were identified. Total four colonies of bacteria were obtained from hive stored pollen of *Apis cerana indica* from the sampling locations. Isolate B1 (from sample PKD-1) and isolate B2 (from sample PKD-3) were obtained from pollen samples collected from Palakkad, whereas isolate B3 (from sample WYD-3) and isolate B4 (from sample WYD-2) obtained from Wayanad. Out of four bacterial isolates, B1 which appeared in red when observed under a compound microscope, inferred that the bacteria were gram negative, while the isolates B2, B3 and B4 which appeared in crystal violet inferred that all three bacteria were gram positive (Fig. 1). Morphological and cultural characters of different

Table 1. Morphological and cultural characters of different bacterial isolates obtained from hive stored pollen of *Apis cerana indica*

Location	Bacterial isolate	Gram reaction	Shape	Color	Margin	Elevation of colony	Form colony
Palakkad	B1	Negative	Rod	Slight yellowish	Irregular	Flat	Round large
	B2	Positive	Rod	Creamy white	Entire	Flat	Round large
Wayanad	B3	Positive	Rod	Creamy white	Entire	Flat	Round large
	B4	Positive	Rod	Creamy white	Entire	Flat	Round large

Table 2. Molecular identification of bacterial isolates obtained from Palakkad and Wayanad districts of Kerala

No.	Sample code	Isolate	Bacteria identified	Accession number	Query coverage (%)	Identity (%)
1	PKD-1	B1	*Pseudomonas aeruginosa*	MH010896.1	100	99.84
2	PKD-2	B2	*Bacillus megaterium*	MN826585.1	100	100
3	WYD-3	B3	*B. megaterium*	KJ500011.1	100	99.69
4	WYD-2	B4	*B. aryabhattai*	MT184818.1	99	100

bacterial isolates obtained from hive stored pollen indicated that, isolate B1 produced large round colonies in the media with irregular margin and flat elevation. Isolates B2, B3 and B4 produced numerous small round colonies with entire smooth margin and flat elevation (Table 1).

When molecular identification of bacterial isolates obtained from Palakkad and Wayanad districts of Kerala were analysed through, 16S rRNA sequencing from the hive stored pollen of *A. cerana indica* revealed the presence of B1 as *Pseudomonas aeruginosa*, B2 and B4 as *Bacillus megaterium* and B3 as *B. aryabhattai* in the hive stored pollens (Table 2).

Total three colonies of fungi were obtained. Isolate T1 (from sample PKD-2) was obtained from Palakkad, whereas isolate W1 (from sample WYD-2) and isolate A1 (from sample WYD-1) were obtained from Wayanad. Morphological and cultural characters of different fungal isolates obtained from hive stored pollen, indicated that, isolate T1 had green large round colonies with entire smooth margin having flat elevation. Isolate W1 had white small round colonies with entire smooth margin having flat elevation. Isolate A1 had orange small round colonies with smooth margin having flat elevation (Table 3).

When molecular identification of fungal isolates obtained from Palakkad and Wayanad were analysed through, ITS sequencing of three fungi resulted T1 as *Trichoderma reesei*, W1 as *Westerdykella multispora* and A1 as *Neurospora crassa* (Table 4).

Kacaniova *et al.* (2012) performed dilution plating analysis for the collected samples and isolated various fungal species like *Penicillium*, *Aspergillus* and *Cladosporium* respectively. Other fungal species like *Botrytis*, *Eurotium*, *Trichoderma*, *Phoma, Fusarium, Mucor, Acremonium, Alternaria* and *Epicoccum* were also identified from the samples.

When microbial load of hive stored pollen samples collected from Palakkad and Wayanad districts of Kerala were analysed, the pollen collected from Wayanad recorded the highest microbial load

Table 3. Morphological and cultural characters of different fungal isolates obtained from hive stored pollen of *Apis cerana indica*

Location	Isolate	Color	Margin	Elevation of colony	Form of colony
Palakkad	T1	Green	Entire	Flat	Round
Wayanad	W1	White	Entire	Flat	Small round
	A1	Orange	Entire	Flat	Small round

($6.6x10^4$ cfu g^{-1}) compared to Palakkad sample ($3.3x10^4$ cfu g^{-1}). From Palakkad, the fungal population varied between $0.6 x10^4$ to $1.3 x10^4$ with a mean of $1 x10^4$ (cfu g^{-1}), whereas the bacterial population varied between $1.3 x10^4$ and $3.3 x10^4$ with a mean of $2.3 x10^4$ (cfu g^{-1}). From Wayanad, the fungal population varied between $3 x10^4$ and $3.6 x10^4$ with a mean of $3.3 x10^4$ (cfu g^{-1}), whereas bacterial population varied between $2.66 x10^4$ and $4 x10^4$ with a mean bacterial population of $3.3 x10^4$ (cfu g^{-1}) (Table 5).

Bonvehi and Jorda (1997) reported that total coliform bacteria population present in the pollen sample ranged from 3-43 cfu g^{-1}. Whereas, *Clostridum, Bacillus* and *Streptococcus* was in the range of 5-10, 5-12 and 5-550 cfu g^{-1}respectively. Kacaniova *et al*. (2012) has reported that total fungal population varied from 2.00×10^2 cfu g^{-1} with an average value of 1.20×10^2 cfu g^{-1} when inoculated on czapek-Dox agar, whereas the fungal population varied from 1.58×10^2 - 9.66×10^2 cfu g^{-1} when inoculated on Malt Extract Agar. Similar study was reported as total fungal population in hive stored pollen samples (Nogueira *et al.,* 2012; Sinacori *et al*., 2013; Thakur *et al.,* 2024).

Total bacterial and fungal population of hive stored pollen of A. cerana indica obtained from Palakkad and Wayanad districts of Kerala: The fungal population of the hive stored pollen samples collected from Palakkad ranged between $0.6x10^4$ to $1.3x10^4$ with a mean of $1x10^4$ (cfu g^{-1}), whereas the bacterial population ranged from $1.3x10^4$ to $3.3x10^4$ with a mean of $2.3x10^4$ (cfu g^{-1}). The fungal population of the hive stored pollen samples from Wayanad ranged from $3x10^4$ to $3.66x10^4$ with a mean of $3.33x10^4$ (cfu g^{-1}). Whereas the bacterial population was in the range of $2.66 x10^4$ to $4x10^4$

Fig. 1 Microscopic image: Gram-negative bacteria

Table 4. Molecular identification of fungal isolates obtained from Palakkad and Wayanad districts of Kerala

No.	Code	Isolate	Fungus identified	Accession no.	Query coverage	Identity
1	PKD-2	T1	*Trichoderma reesei*	MW048853.1	87%	96.23%
2	WYD-2	W1	*Westerdykella multispora*	MH857224.1	100%	99.79%
3	WYD-1	A1	*Neurospora crassa*	MT557113.1	100%	100%

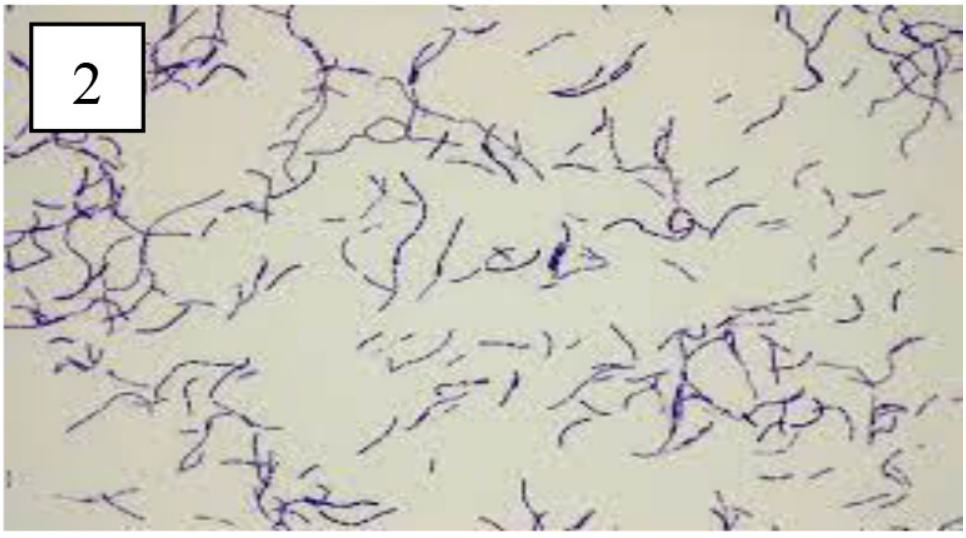

Fig. 2 Microscopic image: Gram-positive bacteria

with a mean of $3.3x10^4$ (cfu g^{-1}). Among the sample from Palakkad, PKD-1 recorded the highest fungal population ($1.3 x10^4$ cfu g^{-1}), followed by PKD-2 ($1x10^4$ cfu g^{-1}) and PKD-3 ($0.6x10^4$ cfu g^{-1}). Similarly, PKD-1 recorded the highest bacterial population ($3.3x10^4$ cfu g^{-1}), followed by PKD-2 ($2.3x10^4$ cfu g^{-1}) and PKD-3 ($1.3 x10^4$ cfu g^{-1}). In Wayanad samples WYD-1 recorded the maximum fungal population ($3.66x10^4$ cfu g^{-1}), followed by WYD-2 ($3.33x10^4$ cfu g^{-1}) and WYD-3 ($3x10^4$ cfu g^{-1}). Similarly, WYD-1 recorded maximum bacterial population ($4x10^4$ cfu g^{-1}) which was followed by WYD-2 ($3.33x10^4$ cfu g^{-1}) and WYD-3 ($2.66 x10^4$ cfu g^{-1}).

The pollen sample data analysis using R software, Welchs's two sample t test, revealed high significant differences among fungal and bacterial population in the samples collected from the hive stored pollen of *A. cerana indica* obtained from Palakkad and Wayanad districts of Kerala (Table 6). In the present study even after 72-96 hours of incubation, actinomycetes and yeast colonies were not recorded on the plated Kenknights and Munaiers medium and Sabouraud Dextrose Agar (SDA) medium.

Table 5. Microbial load of hive stored pollen samples collected from Palakkad and Wayanad districts of Kerala

Location	Load (cfu g^{-1})	t value	p value
Palakkad	$3.3 x10^4$	-4.7295	4.413e-05
Wayanad	$6.6 x10^4$		

Pseudomonas is an inhabitant of bee pollen, contributing to the decomposition of pollen walls, which is commonly detected in honey (Stavropoulou *et al.,* 2023). *Bacillus* sp. possesses the antimicrobial property, which may contribute to

Table 6. Welch two sample t-test statistical analysis of fungal and bacterial population

Location	Colony (cfu g^{-1})	
	Fungi	Bacteria
Palakkad	1×10^4	2.3×10^4
Wayanad	3.3×10^4	3.3×10^4
t value	-5.7155	-2
p value	4.788e-05	0.06473

regulation of host's microbiota as a beneficial microbe or probiotic (Jia *et al.,* 2020). Comparative analysis of microbial communities in the examined honey samples revealed that their major taxa were either indigenous microbiota of honeybees, mostly beneficial and to a lesser extent potential foodborne and human pathogens and/or inhabitants of bee pollen and flowering plants, including both plant protective and phytopathogenic microorganisms.

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A new species of *Zographetus* Watson, 1893 (Lepidoptera, Hesperiidae, Hesperiinae) from the Western Ghats, Peninsular India

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ABSTRACT: A new species of *Zographetus* Watson, 1893 (Lepidoptera, Hesperiidae, Hesperiinae) is described from the Western Ghats of Peninsular India. *Zographetus mathewi* Sadasivan and Kochunarayanan, **sp. nov.,** fits in the *Z. satwa* species-group based on the forewing venation, with vein Cu_2 arising much closer to the wing base than vein Cu_1, and the basal portion of vein Cu_2 and the cubitus up to the origin of Cu_1 swollen. Field images of adults, illustrations of genitalia and information on the early stages of this species are also provided. The larval host plant was identified as *Aganope thyrsiflora* (Fabaceae). A key to the species of the Oriental genus *Zographetus* is also provided.
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KEY WORDS: Biodiversity hotspot, skipper, butterfly, Kerala, Agasthyamalais

INTRODUCTION

Zographetus Watson, 1893 (Lepidoptera, Hesperiidae), limited to the Oriental Region, is represented by 14 species (Fan *et al.*, 2007; Xue *et al.*, 2019; Karmarkar *et al.*, 2021). The currently known distribution of the genus is confined to peninsular India, northeastern India, the Andaman Islands, Indo-China, China, Malay Peninsula, Sumatra, Java, Lesser Sunda Islands, Borneo,

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Palawan, Philippines and Sulawesi (Vane-Wright and de Jong, 2003). These are generally rare skippers and the larvae feed on Gnetaceae, Poaceae, Sterculiaceae and Fabaceae; this polyphagous nature may reflect the morphological heterogeneity of the genus (Vane-Wright and de Jong, 2003; Anonymous, 2023). Members of *Zographetus* may be classified into two species-groups based on male wing venation (position of the origin of vein Cu_2) and secondary sexual characters (swollen veins) (Xue *et al.*, 2019; Fan *et al.*, 2007). The first group is characterised by the forewing vein Cu_2 arising much closer to the wing base than vein Cu_1, and the basal portion of vein Cu_2 with the cubitus swollen in the males (*Z. satwa* species-group); the second is composed of species with forewing vein Cu_2 arising midway or closer to vein Cu_1 than to the wing base, and males without swollen veins (*Z. ogygia* species-group) (Corbet and Pendlebury, 1992; Fan *et al.*, 2007; Xue *et al.*, 2019). The following species belong to the *Z. satwa* species-group: *Z. satwa* (de Nicéville, [1884]), *Z. abima* (Hewitson, 1877), *Z. pallens* de Jong & Treadaway, 1993, *Z. rama* (Mabille, 1876), and *Z. rienki* Xue, 2019. The *Zographetus ogygia* species-group currently includes *Z. ogygia* (Hewitson, [1866]), *Z. doxus* Eliot, 1959, *Z. durga* (Plötz, 1884), *Z. flavalum* de Nicéville, 1887, *Z. hainanensis* Fan and Wang, 2007, *Z. ogygioides* Elwes & Edwards, 1897, *Z. kutu* Eliot, 1959, and *Z. pangi* Fan and Wang, 2007 (Corbet and Pendlebury, 1992; Fan *et al.*, 2007; Xue *et al.*, 2019).

From India, four species of *Zographetus* are currently known: *Z. dzonguensis* Kunte, Karmakar & Lepcha, 2021; *Z. flavalum*; *Z. ogygia*; and *Z. satwa* (Eliot, 1959; Evans, 1932, 1949; Corbet and Pendlebury, 1992; Karmarkar *et al.*, 2021). *Zographetus flavalum,* earlier considered a synonym of *Z. ogygia* by Evans (1949), was resurrected by Eliot (1959) and is known only from Sikkim, its type locality; *Z. dzonguensis* is also known only from its type locality in north Sikkim (Karmarkar *et al.*, 2021); *Z. ogygia* is known from the Western Ghats (WG), Eastern Himalaya, NE India and Andamans, extending into southeast Asia, Sumatra, Java and Borneo; and *Z. satwa* is distributed from Kumaon eastward into the Himalayas and NE India, extending into southeast Asia, Sumatra and Java (Bell, 1925; Evans, 1932, 1949; Corbet and Pendlebury, 1992). Thus, only one species, *Z. ogygia* (Hewitson, [1866]), was known from the WG of Peninsular India. The only available historical record of this taxon from WG is traceable to Bell (1925), where a single specimen was caught in Kanara (in present-day Karnataka state) near the seacoast in the semi-evergreen forest at approximately 800 ft above sea level (ASL) during the monsoon, and it was identified by de Nicéville. Evans (1932) recorded it as *Z. ogygia ogygia* (Hewitson, [1866]) from southern India and its status was mentioned as being rare. However, this taxon found no mention in Evans (1949), Larsen (1988) or Gaonkar (1996) for the WG.

In recent years, there have been plenty of records of *Z. ogygia* from Kerala, Karnataka and Goa in southern India (Anonymous, 2023). During fieldwork in the WG of Kerala state, larvae of a skipper butterfly were noticed on the leaves of the liana *Aganope* (Fabaceae), resembling *Z. ogygia.* A detailed study of the eclosed adult specimens revealed that this taxon differed considerably from *Z. ogygia* in terms of wing venation and shape of the male genitalia. This taxon is described here as new to science, with images of adults, illustrations of male and female genitalia, and details of its early stages. A key to all known species of the Oriental genus *Zographetus* is also provided in this study.

MATERIALS AND METHODS

The taxonomy of the genus *Zographetus* follows Evans (1932, 1949), Corbet and Pendlebury (1992), Fan *et al.* (2007), Xue *et al.* (2019) and Karmarkar *et al.* (2021). Photographs of the pinned specimens and larvae were taken with a Canon EOS 70D DSLR fitted with a 180mm macro lens and an MPE 65 f 2.8 1–5x lens. Details of the early stages were noted after rearing field-collected larvae in the laboratory. The genitalia were dissected after overnight soaking of the severed tip of the abdomen in KOH (10%), studied under a stereo-zoom microscope (HEADZ Model HD81), and preserved

in glycerol. Illustrations were drawn by KS using the stereo-zoom microscope and then digitised. The length of the forewing (FW) was measured as the longest straight-line distance from the wing base to the wing tip, following Van Hook *et al.* (2012). The terminology for wing markings follows Evans (1932) and Corbet and Pendlebury (1992) for descriptions of the genitalia.

Abbreviations used

FW	Forewing
HW	Hindwing
TNHS	Travancore Nature History Society
UpF	Upperside of the forewing
UnF	Underside of the forewing
UpH	Upperside of the hindwing
UnH	Underside of the hindwing
WG	Western Ghats
WLS	Wildlife Sanctuary
ZSI	Zoological Survey of India

RESULTS AND DISCUSSION

Systematic account

Zographetus Watson, 1893

Zographetus Watson, 1893: 72, 84 (Type species: *Isoteinon satwa* de Nicéville).

Gehenna Watson, 1893: 92, 108 (Type species: *Hesperia abima* Hewitson).

Diagnosis of genus: *Zographetus* is a heterogenous genus characterised by palpi with the third palpomere short; FW vein M_2 downcurved at base; with ground colour on upper side dark brown with small white hyaline spots; UnH with ground colour ferruginous yellow, and black spots in cell and post-discal area. Based on forewing venation and secondary sexual characters, two species-groups are recognised. In the *Z. satwa* group, the FW vein Cu_2 arises much closer to the wing base than to vein Cu_1, and the basal portion of vein Cu_2 and the cubitus are swollen in the males (Corbet and Pendlebury, 1992; Fan *et al.*, 2007).

Zographetus mathewi Sadasivan and Kochunarayanan, sp. nov. (Fig. 1, 2A, B, 3, 4A–F)

LSIDurn:lsid:zoobank.org:act:E6594FA0-9A45-4405-86B3-65B174E789B3

Type material: *Holotype* (Fig. 2A, B). ♂ **TLRG # 1005**, Kottiyoor, Kannur District (Coorg landscape), Kerala State, India; col. KS; 15. vi. 2021, 200m ASL, from a private homestead; dry pinned specimen eclosed from reared larvae ex *Aganope thyrsiflora* (Benth.) Polhill (Fabaceae) deposited in the insect collections of ZSI, Kozhikode, Kerala, India (No. ZSI/WGRC/IR/INV.29707).

Paratypes (n=3) (Fig. 2C, D). ♀ **TLRG # 1006**, Kallar, Thiruvananthapuram District (Agasthyamalai landscape), Kerala State, India; col. KS; 15. vi. 2021, 100m ASL, from a private homestead; dry pinned specimen eclosed from reared larvae on *Aganope thyrsiflora* (Fabaceae), deposited in the insect collections of ZSI, Kozhikode, Kerala, India (No. ZSI/WGRC/IR/INV.29708). ♂, **TLRG # 1007**, bearing the same data as TLRG 1006, wet specimen, will be retained in the insect collections of TNHS Thiruvananthapuram, Kerala, as a voucher specimen. **TLRG # 1008**, bearing the same data as the holotype, a dry pinned specimen eclosed from reared larvae, will be deposited in the insect collections of TNHS as a voucher specimen.

Description of the holotype: *Head, thorax and abdomen:* Head, palpi, thorax, femur, coxae, and abdomen clothed with ochre-yellow hairs; hairs around distal thorax and abdomen with greenish hue; legs yellow inside, brownish-yellow externally, joints darker; eyes dark brownish-black; antennae half as long as costa, black, inner aspect chequered with white, club black with a well-defined white patch superomedially, apiculus black (Fig. 2A, B).

FW. (Fig. 2A, B; 4A). Length 14 mm. *UpF*: Ground colour dark chocolate-brown; basal half of costa thickly bordered with yellowish-ochre scales, rest of costa very thinly so; space 1b (cell 1A+2A) clothed with very long golden-orange hairs; a spot in space 2 (CuA_1), just before middle of space 2, quadrate; small spot in 3 (cell M_3) well before middle of space 3, closer to its base, but not occupying its origin; a smaller lower cell-spot; a small post-discal

spot in basal space 6 (cell R_5). Lower cell-spot and spot in 3 are separated by a distance distinctly less than length of spot in 2. Spot in 2 largest, followed by that in 3, then lower cell and one in space 6. Cilia indistinctly chequered with dark and pale purple-grey. Venation with basal portions of FW vein Cu_2 and cubitus swollen up to origin of Cu_1; FW origin of vein Cu_2 closer to wing base than to vein Cu_1; M_3 origin midway between origins of M_2 and Cu_1. *UnF*: In addition, a thick tuft of specialised appressed long black hairs at base of space 1a and 1b, tuft occupies basal margin of dorsum in space 1b, clads origin of vein 1(A_{1+2}) and cubitus till level of origin of vein 2 (Cu_2). Ground colour bright ochreous-yellow, post-discal area and submarginal area purple-brown, post-discal spot in 6 pale yellowish white and bracketed in dark purplish scales; other spots as in UpF.

HW. UpH: Ground colour dark chocolate-brown, cell bearing long brownish-green hairs, unspotted. *UnH*: Ground colour bright ochreous-yellow, post-discal area and submarginal area purple-brown, dark basal spot, and a discal spot in space 7 at its middle and a spot in cell brownish; post-discal series of purple spots from spaces 1b–6; a submarginal series of obscure oval yellowish-ochre spots from 1b–6; cilia checkered with dark and pale purple-grey (Fig. 2 A, B).

Male genitalia. (Fig. 4B–F). Vinculum and associated structures are shown in Fig. 4B; saccus relatively short, almost slightly bent upward (Fig. 4 B). Basal third of valva with dorsal margin shallowly convex, ventral margin sinuous; distal two-thirds constricted and curved upwards; dorsal margin regular, devoid of any teeth, extending posterolaterally into a blunt digitiform process, ventral margin curved upwards ending in a blunt tip (Fig. 4D). In posteroventral view, the outer arm of the vinculum is short and rudimentary, and the inner lamina is small and carries fewer large spines, roughly arranged in two rows around periphery, with a distinctly shaped clasp (Fig. 4B–F). Aedeagus, dorsal margin of distal portion shallowly concave, ventral margin of distal half convex in lateral view, tip downcurved (Fig. 4E).

Description of female:

Head, thorax and abdomen: Head, palpi, thorax, femur, coxae, and abdomen as in males; antennae half as long as costa, black, inner aspect chequered with white, club black, apiculus brown (Fig. 2C, D).

Wings. FW. Length 14 mm. *UpF*: Ground colour dark chocolate-brown; basal half of costa thickly bordered with yellowish-ochre scales, rest of costa very thinly so; space 1b (cell 1A+2A) clothed with very long orange-golden hairs; a small yellowish space streak in space 1at middle; a long rectangular whitish spot in space 2 (CuA_1), just before middle of space 2; small spot in 3 (cell M_3) well before its middle, closer to its base, but not occupying its origin; two small cell spots mirroring each other; a small post-discal spot in basal space 6 (cell R_5) and very small one above it in space 7; lower cell-spot, a spot in 3 separated by a distance almost equal to length of spot in 2. Spot in 2 largest, followed by that in 3, then one in space 6, followed by spot in space 1. Cilia indistinctly chequered with dark and pale purple-grey. *UnF*: Ground colour chocolate-brown, post-discal area and submarginal area ferruginous-brown; spots as in UpF; thick tuft of specialised appressed long black hairs at base of space 1a and 1b as in males. *HW. UpH:* Ground colour chocolate-brown, cell bearing long brownish-green hairs, unspotted. *UnH*: Ground colour bright ochreous-yellow, post-discal area and submarginal area purple-brown, dark basal spot, and a discal spot in space 7 at middle and a spot in cell brownish; post-discal series of purple spots from spaces 1b–6; a submarginal series of obscure oval yellowish-ochre spots from 1b–6; cilia checkered with dark and pale purple-grey.

Female genitalia (Fig. 4G). In posteroventral (end-on view) papillae anales triangular, completely sclerotised, except for posteroinferior border, outer lamina covered with moderately long, sparse hairs; lamella ante-vaginalis wide, trapezoid, mid-ventral margin sharply curved like a keel; lamella post-vaginalis sclerotised, quadrangular, with a well-defined lateral triangular plate on each side from which arise, on each side, a long, curved flat spine; ductus bursae tube-like, short and sclerotised.

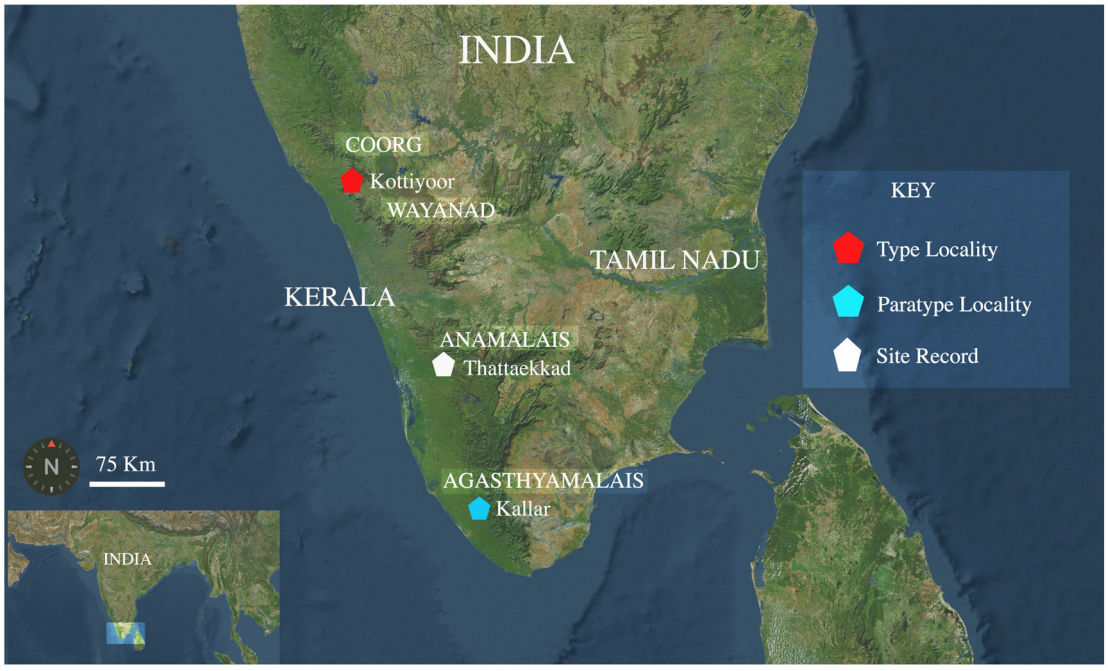

Fig. 1 Map showing the site records of *Zographetus mathewi* **sp. nov.**

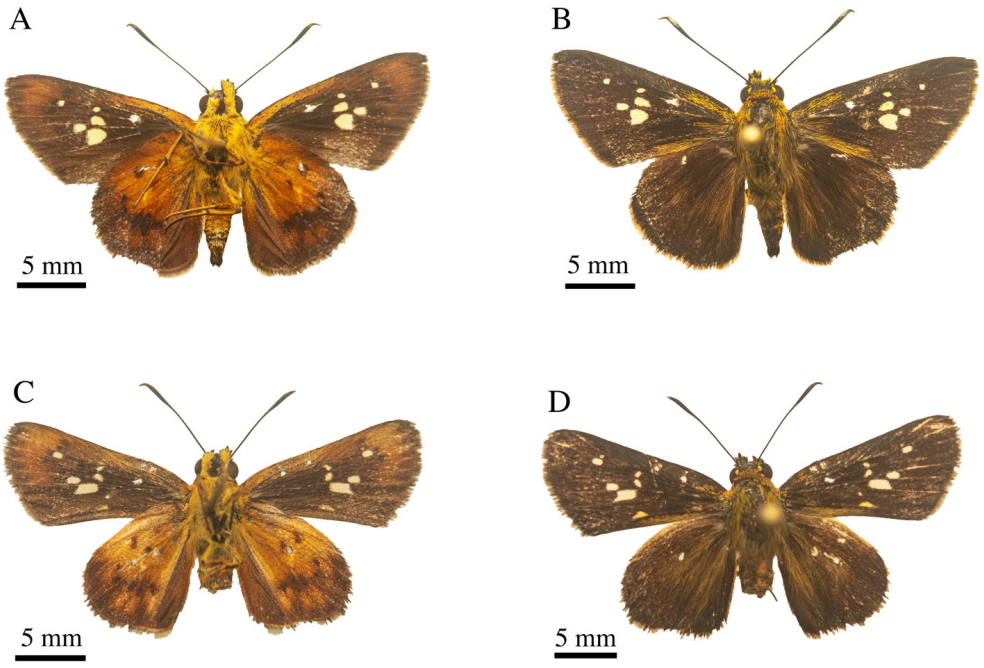

Fig. 2 *Zographetus mathewi* **sp. nov.**, Holotype, and paratype (tip of abdomen removed for dissection). A–holotype male ventral view; B–holotype male dorsal view; C–paratype female ventral view; D–paratype female dorsal view. © Kalesh Sadasivan

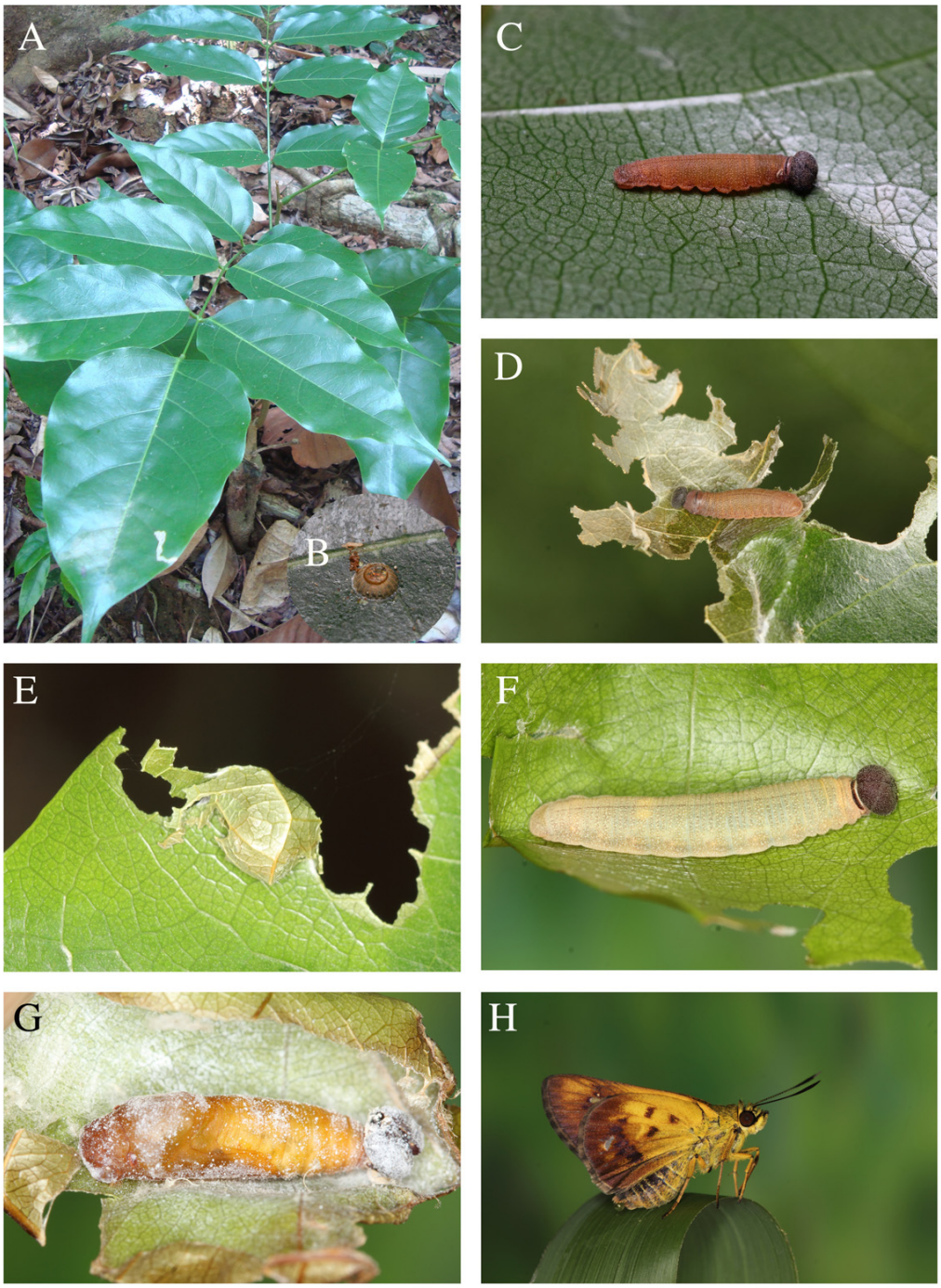

Fig. 3 *Zographetus mathewi* **sp. nov.**, Lifecycle and hostplant. A–*Aganope thyrsiflora* (Benth.) Polhill (Fabaceae); B–egg; C–larva; D–intermediate (third) instar larva with the shelter opened showing fir-tree appearance; E–spherical larval shelter in situ; F–final instar larva; G–pupa; H–adult butterfly. © Baiju Kochunarayanan

Variation: The upper cell-spot on UpF in some specimens was highly reduced or absent in males. The quadrangular spot in space 2 is occasionally higher than broad but never longer than high. A small apical spot may occasionally be observed in the UnF space 7.

Etymology: The new species is named after Dr. George Mathew, an Indian lepidopterist and former Head of the Entomology Division at the Kerala Forest Research Institute (KFRI), Peechi, Thrissur, Kerala, for his significant contributions to lepidopteran and forest insect research in the state. The proposed common English name is Sahyadri Spotted Flitter.

Distribution: The butterfly is seen in the semi-evergreen, moist deciduous, and West-coast Evergreen forests of Kerala in all landscapes below 600 m. Site-specific personal records of larvae are from Aralam and Kottiyoor (Kannur District), Nilambur (Malappuram District), Edamalayar-Pooyamkutty (Ernakulam District), Shendurney (Kollam District), Kallar-Ponmudi Valley, Bonaccord, Peppara WLS, and Neyyar WLS (Thiruvananthapuram District). Adults are extremely rare in the field, and the only record from our side is from Edamalayar in the Lower Periyar landscape from a West-Coast Tropical Evergreen Forest in March 2022 (JJ and TA). To the best of our knowledge, this new species is endemic to the WG.

Ecological notes and early stages (Fig. 3): The eggs are laid on the adaxial surface of the leaf, usually on mature leaves. They never use fresh or immature leaves. The newly hatched larva cuts the margin of the leaf and makes a small cell (larval shelter). When opened, the mature larval cell (larval shelter) has a fir-tree appearance, and when folded with larval silk, it creates a spherical cell. Feeding occurs around the leaf blade near the cell and has a moth-eaten appearance. The total duration of the larval stages is 21–26 days, the final instar lasts 5–6 days, and the pupal period is 10–11 days. The new species feeds on *Aganope thyrsiflora* (Benth.) Polhill (Fabaceae), as far as is known (Fig. 3A). The larvae of *Z. ogygia* and *Z. doxus* occur on *Archidendron jiringa* (Jack) Nielsen (Fabaceae), *Z. rama* is recorded on *Gnetum* (Gnetaceae), *Z. abima* on *Pterospermum* (Sterculiaceae) as per Igarashi & Fukuda (2000) and Eliot *et al.* (2006). Due to the paucity of published data on the early stages of other known species of *Zographetus*, finer comparisons of larval morphology could not be performed.

DISCUSSION

Zographetus mathewi **sp. nov.** is distinguished from all the other members of the genus by the combination of the following characters – forewing dorsal side with basal portions of vein Cu_2 and cubitus swollen, this glandular swelling of cubitus long, reaching more than halfway to vein Cu_1; origin of vein Cu_2 closer to wing base than to vein Cu_1; spot in space Cu_1 (space 2) is broad, quadrate in males, rectangular in females; distance between lower cell-spot and the spot in 3 is always less than the length of the spot in 2; ventral side of forewing with a basal black hair tuft; hindwing ventral side with yellow-ochre super-scaling not condensed into a band; the dark discal spots well-defined, with the black spot in space 7 at its middle.

Relationships of the new species to currently recognised *Zographetus* species-groups

1. Affinity of *Z. mathewi* sp. nov. to *Zographetus* species-groups based on wing venation

Based on forewing venation and secondary sexual characters, the genus includes two distinct species-groups proposed by Eliot (1992), followed by Fan *et al.* (2007). One group has forewing vein Cu_2 arising much closer to the wing base than to vein Cu_1, and the basal portion of vein Cu_2 and the cubitus are swollen in the male (*Z. satwa* species-group); the second is composed of the remaining species, with forewing vein Cu_2 arising slightly closer to vein Cu_1 than to the wing base, and the male without a swollen vein (*Z. ogygia* species-group). Species-group classifications, though somewhat artificial, may be practically useful in segregating and differentiating species within a genus with ease.

***Zographetus satwa* species-group.** *Zographetus abima*, *Z. pallens*, *Z. rama*, *Z. rienki*, and Z. *satwa* belong to the *Z. satwa* species-group. Based on the venation, *Z. mathewi* **sp. nov.** falls into the *Z. satwa* group, with its FW vein Cu_2 arising much closer to the wing base than to vein Cu_1, M_3 origin midway between the origins of M_2 and Cu_1, and the basal portions of vein Cu_2 and cubitus being swollen. The species in the *Z. satwa* species-group are easily distinguished from *Z. mathewi* **sp. nov**.

The species *Z. abima* from Sulawesi (Celebes) is similar to the new species but is diagnosed by UnH with dark discal spot in space 7 well beyond its middle; UpF lower cell-spot and the spot in 3 separated by a distance almost equal to the length of the spot in 2. In the new species, the UnH dark discal spot in space 7 is at its middle, and the FW lower cell-spot and the spot in 3 are separated by a distance distinctly less than the length of the spot in 2. The UnH is overlaid with yellow scales condensed into a broad, pale-yellow band, whereas no such band exists in *Z. mathewi* **sp. nov**. The long glandular swelling of cubitus, reaching more than halfway to vein $Cu_{1,}$ and a broad, quadrate spot in space Cu_1 (space 2) in *Z. mathewi* **sp. nov**., eliminates *Z. rama* which has UnF with glandular swelling of cubitus short, extending only one-third of the distance to vein Cu_1; UpF with spot in space Cu_1 (space 2) narrow, higher than wide; yellow scaling pale. The well-marked UnH with well-defined dark spots differentiates *Z. mathewi* **sp. nov**., *Z. rienki* has UnH unmarked and the yellow scaling dull and sparse, while the UnH marked with dark spots; UnH yellow scaling is of an ochreous shade in *Z. mathewi* **sp. nov**. *Zographetus satwa* lacks the UnF black hair tuft seen in *Z. mathewi* **sp. nov**.

***Zographetus ogygia* species-group.** Basal portions of FW vein Cu_2 and cubitus not swollen; FW origin of vein Cu_2 is variable in *Z. ogygia* species-group as defined here (see key below). The *Z. ogygia* group currently has *Z. doxus, Z. durga, Z. flavalum, Z. hainanensis, Z. ogygia, Z. ogygioides, Z. kutu,* and *Z. pangi.* All the species mentioned above falling in the *Z. ogygia* group are easily differentiated from Z. *mathewi* **sp. nov.,** by the absence of swollen FW veins. In addition, *Z. doxus*, *Z. durga, Z. flavalum, Z. ogygia*, *Z. ogygioides,* and *Z. kutu,* differ from the new species by their males with FW vein Cu_2 arising closer to vein Cu_1, while in the new species, the FW vein Cu_2 arises much closer to the wing base than to vein Cu_1. The species *Z. flavalum* lacks the sub-uncal processes (Eliot, 1959), which are present in *Z. mathewi* **sp. nov.**, and other congeners.

The species *Z. dzonguensis* could not be placed in the key (see below) as the critical taxonomical details of FW venation, such as the swollen or non-swollen state of the basal portions of Cu_2 and cubitus, the origin of veins Cu_1 and Cu_2, and the status of the paired sub-uncal processes, are missing in the description in Karmarkar *et al.* (2021). However, *Z. dzonguensis* is easily distinguished from Z. *mathewi* **sp. nov.,** by the very prominent cell-spot UnH, the broad brown margins on both wings on the underside, and the absence of discal spots in mid-space 7 UnH in the former. Moreover, the structure of the male genitalia is very different in these two species, as depicted by Karmarkar *et al.* (2021).

2. *Affinity of Z. mathewi* sp. nov. to *Z. ogygia* species-complex based on the structure of male genitalia

Evans (1949) mentioned four species in his work– *Z. satwa*, *Z. ogygia, Z. rama* and *Z. abima*. But, considered that there was only one real species in the *Z. ogygia* (Evans 1949, op. cit., p. 300), and lumped all the described forms into four subspecies of *Z. ogygia, viz. Z. ogygia* with Z. *flavipennis* (de Nicéville, 1885) and *Z. flavalum* de Nicéville, 1887 listed as synonyms; *Z. andamana* Evans, 1926, *Z. durga*, and *Z. ogygioides*. Eliot (1959) revised this *Z. ogygia* species-complex of Evans (1949) into two forms based on the structure of male genitalia. The structure of the clasp (valve) and the paired sub-uncal processes with a narrow outer arm and a medial dentate lamina were the key characters. One form which belongs to the types of *Z. ogygia* has the outer arm always short,

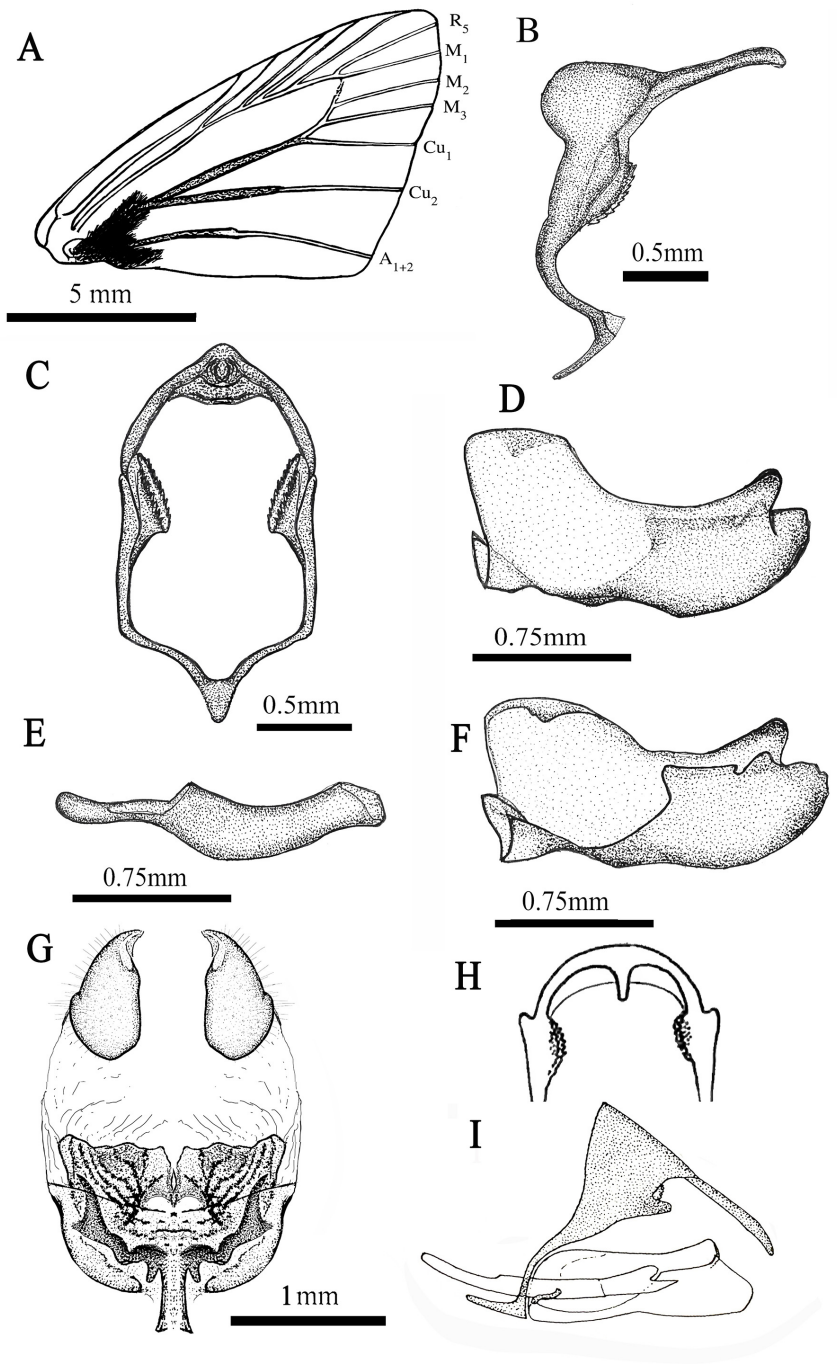

Fig. 4 *Zographetus mathewi* **sp. nov.**, Illustrations. A–forewing venation of a male showing the swollen veins at basal portion of vein Cu_2 & cubitus up to origin of Cu_1, and specialized basal black hair tuft at base; B–lateral view of vinculum and associated structures; C–end-on view of the uncus, showing sub-uncal processes, vinculum and associated structures; D–left valve (clasp) lateral view; E–aedeagus; F–right valve (clasp) medial view; G–ventral end-on view of female genitalia showing the sclerotized triangular anal papillae, trapezoid lamella ante-vaginalis, well-sclerotized lamella post-vaginalis with long lateral spines; H–end-on view of the male uncus, showing sub-uncal processes of *Z. ogygia* redrawn from Eliot (1959) (not to scale); I–male genitalia of *Z. ogygia* (left valve removed) based on Corbet & Pendlebury (1992) (not to scale). © Kalesh Sadasivan

the inner lamina small and carrying comparatively few large spines, usually roughly arranged in two rows around the periphery; and a second form has the outer arm of variable length, though usually longer than the other form, with the inner lamina larger and covered almost all over with numerous small teeth, seen in *Z. doxus*.

It was found that the genitalia of the new species is similar to those of the first form of the *Z. ogygia* species-complex described by Eliot (1959) (Fig. 4C). However, the new species differs from *Z. ogygia*, with which it was previously confused in the WG, by the following characteristics: FW vein Cu_2 arising much closer to the wing base than to vein Cu_1, no bands on UpF in males, and the basal portions of vein Cu_2 and cubitus being swollen. The male genitalia of *Z. ogygia* is very different from those of *Z. mathewi* **sp. nov.** On an end-on view of the uncus, *Z. ogygia* has the outer arm always short, the rounded inner lamina small and carrying comparatively fewer large spines, usually roughly arranged in two rows around the periphery (Fig. 4H), while *Z. mathewi* **sp. nov.** completely lacks the outer arm, and the inner lamina is triangular, bearing small teeth in two rows (Fig. 4C). The structure of the clasp is very different in *Z. ogygia* (Fig. 4I), and Z. *mathewi* **sp. nov.** (Fig. 4D, F).

Key to known species and species-groups of the genus *Zographetus* Watson, 1893

The key is based on Corbet and Pendlebury (1992), Jong and Treadaway (1993), Fan *et al.* (2007), Xue *et al.* (2019) and Karmarkar *et al.* (2021). The species *Z. dzonguensis,* though distinct, could not be placed in the key as the critical details of FW venation and the status of the paired subuncal processes are lacking in the original description in Karmarkar *et al.* (2021).

1. Basal portions of FW vein Cu_2 and cubitus swollen; FW origin of vein Cu_2 closer to wing base than to vein Cu_1 (*Z. satwa* species-group) 9

– Basal portions of FW vein Cu_2 and cubitus not swollen; FW origin of vein Cu_2 variable (*Z. ogygia* species-group)..... 2

2. UpF without secondary sexual characters; FW vein Cu_2 arises closer to vein Cu_1 than to the wing base 7

– UpF with secondary sexual characters; FW origin of vein Cu_2 variable..... 3

3. UpF with an orange hair tuft at basal dorsum; FW vein Cu_2 arises much closer to the wing base than to vein Cu_1..... 4

– UpF with special brands; FW vein Cu_2 arises closer to vein Cu_1..... 5

4. Upper cell-spot larger and distinct; space M_2 unmarked dorsally but black ventrally; subzonal sheath of aedeagus bent (China)..... .....*Z. pangi* Fan and Wang, 2007

– Upper cell-spot smaller; spot in space M_2 white on both sides; subzonal sheath of aedeagus straight (China).....*Z. hainanensis* Fan and Wang, 2007

5. UnH basal two-thirds dusky sulphur with a dark spot in cell and space $Sc+R_1$; outer third purple-brown, more or less incorporating the dark discal spots; brands vestigial or inconspicuous; outer arm of subuncal process well-developed (Philippines) .....*Z. durga* (Plötz, 1884)

– UnH uniformly coloured, with a series of dark spots in spaces Cu_2 to $Sc+R_1$ (in space Rs spot may be vestigial) and in cell..... 6

6. UnH golden brown to rich ferruginous; male genitalia with outer arm of subuncal process vestigial (Sikkim to Java)..... .....*Z. ogygia* (Hewitson, [1866])

– Discal area on UnH yellowish in males, most of the wing yellowish in females; outer arm of subuncal process more or less developed (Thailand to Borneo) ……..…… *Z. doxus* Eliot, 1959

7. FW with three spots in subapical and one spot at lower half of distal cell; spot in space M_3 elongated, overlapped with that in Cu_1 and adjacent to cell-spot (Sikkim) ....*Z. flavalum* de Nicéville, 1887

– FW with one subapical spot and two separated

cell spots; in males, spot in space M_3 small, not overlapping with that in Cu_1 and detached from cell-spot..... 8

8. Small insect, FW 13 mm; UnH post-discal spots well marked (Thailand to Sumatra)..... .....*Z. ogygioides* Elwes & Edwards, 1897

– Larger insect, male FW 16 mm; FW cell spots subequal; UnH very dark ferruginous, more or less purple washed, spotting faint or absent (Malaysia).....*Z. kutu* Eliot, 1959

9. UnF with a basal black hair tuft; no brands on male UpF.....10

– UnF without black hair tuft, dorsal side of forewing with special brands on either side of sub-basal portions of veins A_{1+2} and Cu_2, and on lower side of cubitus (Sikkim to Java)…….....…….… .....*Z. satwa* (de Nicéville, [1884])

10. UnH overlaid with yellow scales condensed into a pale yellow broad band from vein Cu_1 (v2) to vein Sc+R (v8); veins swollen in the basal part only (Philippines)…….....……...…... .....*Z. pallens* de Jong & Treadaway, 1993

– Yellow super-scaling on UnH not condensed into a band…….....…..... 11

11. UnF with glandular swelling of cubitus short, extending only one-third of the distance to vein Cu_1; UpF with spot in space Cu_1 (space 2) narrow, higher than wide; yellow scaling pale (Burma to Philippines and Sulawesi)…..... *Z. rama* (Mabille, 1876)

– UnF with glandular swelling of cubitus long, reaching more than halfway to vein Cu_1 and spot in space Cu_1 (space 2) broad, quadrate, or broader than high……..... 12

12. UnH not marked with dark spots; UnH yellow scaling dull and sparse (China)…….....….. .....*Z. rienki* Xue, 2019

– UnH marked with dark spots; UnH yellow scaling ochreous..... 13

13. UnH dark discal spot in space 7 well beyond its middle; lower cell-spot and the spot in 3 separated by a distance almost equal to the length of the spot in 2 (Sulawesi)..... *Z. abima* (Hewitson, 1877)

– UnH dark discal spot in space 7 at its middle; lower cell-spot and the spot in 3 separated by a distance distinctly less than length of spot in 2 in males (WG in Peninsular India) .....…..… ……..... *Z. mathewi* **sp. nov.**

The discovery of a new species of butterfly from the WG, despite more than 200 years of butterfly exploration, highlights the richness of this biodiversity hotspot remaining unexplored. The superficial resemblance of the species to *Z. ogygia* is interesting and thus reiterates the need to examine male genitalia for accurate species diagnosis in skipper butterflies. The previous records of *Z. ogygia* from this region need to be reassessed in light of this discovery. The genus *Zographetus* comprises a heterogeneous group of species with differences in forewing venation and secondary sexual characters. The heterogeneity in adult morphology and the polyphagous nature of the larvae of this genus demand a detailed morphological and phylogenetic analysis to infer lineages and the validity of species-groups within it.

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A new species of *Lyriothemis* Brauer, 1868 (Odonata, Libellulidae) from the Western Ghats of Peninsular India

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ABSTRACT: A new species of phytotelma-breeding dragonfly, *Lyriothemis abrahami* **sp. nov.**, is described from the Western Ghats of Peninsular India. The new taxon was previously misidentified as *L. flava* Oguma, 1915 (= *L. tricolor* Ris, 1916). In contrast to *L. flava,* the new species is sexually dimorphic, with males easily identified based on the characteristic shape of the hamules. Females are identified by their jet-black and yellow colour with triangular yellow streaks on the dorsum of the abdomen and the distinct triangular shape of the vulvar lamina. Photographs of mature adults, tenerals, hamules, illustrations of vesica seminalis, vulvar lamina, and a key to *Lyriothemis* of the Indian subcontinent are provided.
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KEY WORDS: Anisoptera, new taxon, taxonomy, dragonfly, phytotelmata, Kerala

INTRODUCTION

The genus *Lyriothemis* Brauer, 1868 comprises approximately 18 species of dragonflies distributed from Sri Lanka and Peninsular India to Northeast India (NE India), Tibet, Myanmar (Burma), and parts of Southeast Asia in the Oriental region, Palearctic East Asia, Sulawesi and New Guinea in the Australasian region (Fraser, 1936; Van der Poorten, 2009; Paulson *et al.,* 2025). The Indian region hosts *L. acigastra* (Selys, 1878), *L. bivittata* (Rambur, 1842)*, L. cleis* Brauer, 1868, and *L. flava* Oguma, 1915 (= *L. tricolor* Ris, 1916), all recorded from NE India; *L. mortoni* Ris, 1919 from

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Myanmar (Fraser 1936); *L. defonsekai* van der Poorten, 2009 from Sri Lanka (van der Poorten, 2009); and the recently described *L. lucifera* Seehausen & Dawn, 2024 from the Andaman Islands (Seehausen and Dawn, 2024). The distribution of this genus in Peninsular India was not mentioned in any previous literature (Fraser, 1936) until Das *et al.* (2013) reported the presence of *L. tricolor* and Emiliyamma *et al.* (2013) recorded *L. acigastra* from the state of Kerala. According to the latest review of Odonata in Kerala by Nair *et al.* (2021), only records of *L. tricolor* and *L. acigastra* are available from the state. Asahina (1961) treated *L. tricolor* Ris, 1916 as a synonym of *L. flava* Oguma, 1922, which was also followed by van der Poorten (2009). However, according to Paulson *et al.* (2025), *L. tricolor* should be considered a synonym of *L. flava* Oguma, 1915, since the name appeared originally in Oguma (1915). Therefore, *L. flava* Oguma, 1915, should be prioritised over *L. tricolor* Ris, 1916. During our studies on the odonates of Kerala, we encountered libellulid larvae in a phytotelma within a homestead in Pooyamkutty, central Kerala, India. The larvae were then collected and reared under laboratory conditions. Larval rearing since 2021 yielded adults morphologically similar to *L. flava* but differed in the structure of male hamules. This prompted us to conduct a detailed morphological examination of freshly collected mature insects. A thorough study, including dissection of males, revealed that the species is new to science and is described herein.

MATERIALS AND METHODS

The collected insects were preserved in absolute alcohol in plastic vials. Morphological terminology follows Garrison *et al.* (2006) and Chao (1990) for genitalia. The standardised species nodal index (SSNI) concept follows Sadasivan *et al.* (2023). The known distribution of species in the WG follows Nair *et al.* (2021). Measurements and morphological details of all species mentioned in the text are based on specimens in the voucher collection of the TORG and those deposited in the ZSI, Kozhikode, Kerala, India. Field photographs were taken using a Canon 70D Digital SLR camera, Canon 180 mm macro lens, and MPE 65 f 2.8 1–5x macro lens. Morphological studies were conducted, and measurements were taken using a HEADZ Model HD81 stereomicroscope. The genitalia and anal appendages were dissected, studied under a stereo-zoom microscope, and preserved in glycerol. The illustrations were hand-drawn and digitised by KS. The larvae collected from phytotelmata were photographed, collected using an insect net, reared, allowed to harden for a week, and then preserved in absolute ethanol. The distribution map was prepared using Apple Maps © Apple Inc.

Abbreviations used

AL	Abdominal length, including the appendages
ASL	Above sea level
Ax	Antenodal crossveins
FW	Forewing
FL	Forewing length
HL	Hindwing length
HW	Hindwing
Pt	Pterostigma
Px	Postnodal crossveins
S1–S10	Abdominal segments
SSNI	Standardised Species Nodal Index
TNHS	Travancore Nature History Society
TORG	TNHS Odonate Research Group
WG	Western Ghats
WLS	Wildlife Sanctuary
ZSI	Zoological Survey of India

RESULTS

Systematic account

Genus *Lyriothemis* Brauer, 1868

Type species *Lyriothemis cleis,* Brauer, 1868

Diagnosis of the genus: Vertex with tubercle bifid; eyes contiguous for a moderately long space; prothorax with small posterior lobe; thorax robust; abdomen rather broad, triquetral, of nearly uniform

width, much shorter than HW, base not thickened, segments 2, 3 carinated, segments slightly perfoliate in female, wings broad, especially HW, moderately long, obtusely rounded at tip, Pt moderate; FW with 16–20 Ax, 9–12 Px, first two Px not continuous, cells of lower postcostal area usually more or less bisected towards apex, beyond Pt, triangle rather short, traversed, on a level with that of HW, outer side generally very oblique, followed first by three rows of cells, then by several rows of two, then by three and more, subtriangular space consisting of three cells, sectors of arculus stalked, one supratriangular nervure before triangle, lower basal cell with two or three cross nervures, nodal sector only slightly waved towards base, cells between nodal and subnodal sectors simple, or only last bisected, these sectors, as well as those of arculus, nearly continuous, but towards extremity very strongly curved downwards to hind margin, almost at a right angle, lower sector of triangle nearly as long as upper, slightly and regularly undulating; HW considerably broader than FW, with 12–13 Ax, 10 Px, first two or three Px not continuous, triangle traversed, followed by two rows of cells, its base corresponding with arculus, one supratriangular nervure occasionally present, three or four nervures in lower basal cell; anal appendages of usual libelluline type nearly as long as eighth segment, lower appendage broad, triangular; genitalia with lamina hood shaped, projecting, foliate with very short inner spine, lobe small, ungulate constricted at base (Kirby, 1889; Fraser, 1936).

***Lyriothemis abrahami* Sadasivan, Jose, Nair & Palot, sp. nov.**

LSIDurn:lsid:zoobank.org:act:A25F1985-E013-4332-8D34-24FB764EB87A

(Figs. 1, 2, 3B, C, E, F, 4A, E, F, 5A, C, E)

Material studied:

Holotype: **TORG # 1053**: ♂, Aaralam WLS, Kannur district, Kerala State; 90m ASL; 20. iv. 2025, K. Sadasivan leg., ex. flight, will be deposited at ZSI Kozhikode, Kerala, India, under the accession number ZSI/WGRC/IR/INV.29705.

Paratypes (3♂♂ and 3 ♀♀). **TORG # 1054**: ♂, Ponmudi, Thiruvananthapuram District, Kerala State; 700m ASL; 17. v. 2025; K. Sadasivan leg. ex. flight, will be deposited at National Centre for Biological Sciences (NCBS), Bengaluru, Karnataka, India. **TORG # 1055**: ♂, bearing the same collection details as TORG # 1054, will be deposited in the voucher collection of the TORG, Thiruvananthapuram, Kerala, India. **TORG # 1056**: ♀, bearing the same collection details as TORG # 1054, will be deposited at ZSI Kozhikode, Kerala, India, under the accession number ZSI/WGRC/IR/INV.29706. **TORG # 1057**: ♂ (teneral) and **TORG #1058**: ♀ (teneral): Pooyamkutty, Ernakulam district, Kerala; 150m ASL; 15. iv. 2025; K. Sadasivan and J. Jose leg., ex larvae, will be retained as voucher specimens in the collection of TORG, Thiruvananthapuram, Kerala, India. **ZSI/WGRC/IR-INV-2659**: ♀ teneral: Anachathapara, Salim Ali Bird Sanctuary, Thattaekkad, Ernakulam District, Kerala, India; 68m ASL; 03. iv. 2013; M.J. Palot leg., deposited at the ZSI Kozhikode, Kerala, India.

Description of the holotype male:

Head (Figs. 2A, B, C, D, E). Labium black, marked with a large yellow spot on antero-superior aspect; labrum, anteclypeus, postclypeus, antefrons yellowish-white, postfrons with squarish patch of metallic dark greenish-black, its sides narrowly yellow; vertex metallic black with metallic blue lustre, with two small sharp tubercles; antennae black; occiput black, suffused with brown posteriorly; labrum, anteclypeus, postclypeus, antefrons, and postfrons medially bearing short brown setae; eyes black with pale yellow-grey patches inferolaterally, dark chestnut-brown dorsally; post-ocular lobe dorsally and posteriorly black, anterolaterally pale yellow with a sharp triangular black extension anteriorly, arising from its thin black posterior border.

Prothorax (Figs. 2A, D, E). Anterior lobe white with a thin black posterior border merging with black middle lobe, whole of posterior lobe black; middle lobe with a pair of paradorsal pale yellow triangular spots; laterally black markings with grey suffusion and a small pale yellow spot on each side.

Synthorax (Figs. 2A, D, E). Black with yellow markings as follows: short, oval antehumeral stripes on mesepisternum not extending halfway up synthorax towards antealar sinus on either side of dorsal carina; large yellow spots on tergum and antealar sinus; mesepimeron with a broad oblong yellow stripe with rounded inferior border, superiorly almost reaching wing base, anterior corner right-angled, posterior angle acutely produced; mesepimeron almost completely yellow with a very thin black border near its anterior suture; legs black, femora with short spines growing progressively longer distally, tibiae with longer black spines near tibiofemoral joint.

Wings (Figs. 3C, E, 4A). Venation as for the genus, HW broad, main veins Riii, IRiii Riv+v, MA curved, hyaline, veins black, bases of FW and HW with mild brown suffusion to the level of arculus. FW with one cubito-anal crossvein, triangle 2-celled, subtriangle 3-celled, discoidal field 3-celled until approximately level of nodus; Pt black, extending 2.5–3 cells, four times longer than wide. HW membranule black-grey, 2 cubito-anal crossveins, triangle 3-celled, supratriangle l2-celled, a well-developed 20-celled anal loop, nodal index of Fw: Ax 17–18, Px 9–10; Hw: Ax 13–14, Px 11–12; SSNI 17.5:9.5/13.5:11.5. Pt black, covering 3–3.5 cells, four times longer than wide.

Abdomen (Figs. 2A, F, G). In dorsal view, thinner than thorax, broadest at S3; ground colour orange-red with each segment delineated with jet-black dorsal, intersegmental and lateral carinal lines; S1 with mid-dorsal crescentic yellow spot, laterally black; S2 black, near its posterior end with a pair of paradorsal triangular orange-brown spots, within it a thin transverse black streak on either side; S3–S7 marked similarly with black dorsal, intersegmental, lateral carinal black lines, rest of segment orange-red, suffused with brown laterally near carina and black colour of each intersegmental line in S5–S8 extending into orange of each segment on their posterior for a variable distance; mid-dorsal black lines spindle-shaped, broader at midsegment, expand as short black transverse streaks near posterior one-fourth of segment; S8 yellow-orange; black dorsal carinal marking wine-glass shaped, black posterior intersegmental border broader compared to previous segments; S9, S10 black. In ventral view, ground colour yellow, each segment marked thickly with mid-ventral, intersegmental black lines and lateral carinal lines; distal half of each segment faintly suffused with black; distal half of S8–S10 completely black.

Secondary genitalia (Figs. 2C; 4C, E, F; 5A). Hamulus black, pentagonal in anteroventral view, higher than broad, not depressed in center, hamular inferolateral corner right-angled, well-defined, medial border with a distinct insinuation at junction of its inferior and middle thirds; inferior border straight, recurved, posterolaterally directed, blunt, finely trifid tip on inferomedial angle; superomedial angle curved. Genital lobe slender, tip blunt, about 4 times as long as wide, pale yellow-brown.

Vesica spermalis (Figs. 5E, 5F). Vesicle (segment 1) moderately bulged, length slightly shorter than combined length of stem (segment 2) and median segment (segment 3); stem (segment 2) with inner margin rugose and straight, outer margin concave and bears a prominent blunt tooth-like distal extension; median segment (segment 3) of same length as stem (segment 2) with convex outer and inner border; flagellum short, half the length of posterior lobe, meeting in midline (in ventral view); posterior lobe broad and long, almost length of median segment.

*Anal appendage*s (Figs. 2H, I, 4B). Cerci brown-black, slightly longer than twice length of S10, with a terminal black spine on each side extending posterolaterally slightly beyond epiprocts; subapically bearing 6–7 small black blunt ventral teeth. Epiprocts of normal libellulid type, yellow-brown dorsally with lateral borders, tip suffused with brown, bearing two short dorsally directed brown-black spines at caudal end.

Measurements (in mm). AL: 35, FL: 42, HL: 40, Pt: 4.0

Description of the paratype female TORG #1056:

Head. Markings same as in males.

Prothorax. Marked as male, except laterally,

Fig. 1 The site records of *Lyriothemis abrahami* **sp. nov.** from the Western Ghats of southern India

middle lobe has two small pale yellow spots on each side, instead of one in males.

Synthorax. Similar to that of males, except with yellow markings. Antehumeral stripes on dorsum of mesepisternum long, extending two-thirds up thorax towards antealar sinus on either side of dorsal carina; anterolateral yellow thoracic band as broad as or narrower than black area posterior to it.

Wings (Fig. 3E). Venation as for male, hyaline, veins black, bases of FW and HW with mild brownish suffusion to level of arculus. FW with one cubito-anal crossvein, triangle 2-celled, subtriangle 2-celled, discoidal field 3-celled till a little distal to level of nodus, Pt black extending 2 cells, 4.5 times longer than wide. HW with two cubito-anal crossveins, triangle 2-celled, supratriangle 1-celled, well-developed 20-celled anal loop, nodal index in FW: Ax 16 and Px 9–10; HW: Ax 11–12, Px 10–11; SSNI 16.0:9.5/11.5:10.5; Px 10–11; Pt black, covering 2.5 cells, 4.5 times longer than wide.

Abdomen. In dorsal view, thinner than thorax, broadest at S3; ground colour jet-black, S1 dorsally and inferolaterally yellow, dorsolaterally black; S2 with paradorsal yellow spots, inferolaterally yellow, distolaterally black; S3 with paradorsal yellow spots, dorsolateral black, anterolaterally yellow; S4–S7 having a pair of paradorsal and lateral yellow triangular spots extending to about middle of segment, paradorsal and lateral spots joined at base in S6–S7, S8–S10 completely black. In ventral view, ground colour yellow, each segment marked thinly with mid-ventral, intersegmental black lines, and lateral carinal lines; distal half of each segment faintly suffused with brown; distal half of S8–S10 black brown.

Vulvar scale (Figs 4D and 5C). Vulvar scale triangular, anterolateral margin straight, posterolateral margin slightly concave, the medial margin notched.

*Anal appendage*s. Cerci brown-yellow basally, distally black; slightly shorter than twice length of S10, with a terminal black pointed spine on each side extending posterolaterally well beyond epiproct. Epiproct basally with a yellow-brown triangular mark on dorsum, with its posterolateral borders yellow; tips bearing pale yellow setae.

Measurements (in mm). AL: 32, FL: 42, HL: 40, Pt: 4.0

Variation. The extent of black markings, especially on the abdomen, varies in males; in some, the

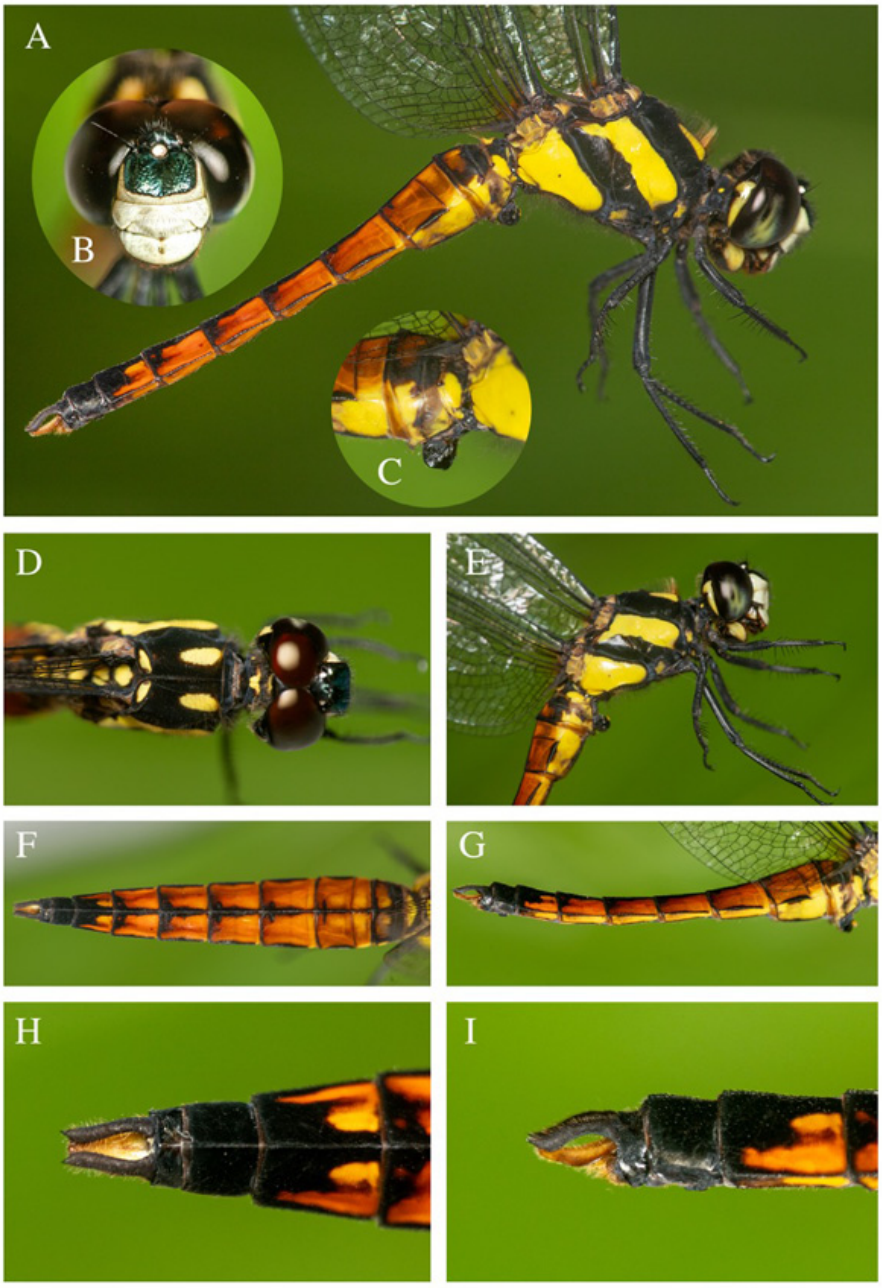

Fig. 2 *Lyriothemis abrahami* **sp. nov.** Holotype TORG # 1053. A–lateral view of the holotype; B–close-up of head; C–lateral view of segment 2; D–dorsal view of head, prothorax and synthorax; E–lateral view of head, prothorax, and synthorax; F–dorsal view of abdomen; G–lateral view of abdomen; H–dorsal view of S8–10, and anal appendages; I–lateral view of S8–10, and anal appendages. © Kalesh Sadasivan

intersegmental lines are thin, while in others, this black extends to a variable distance into the orange of each segment on its posterior part. In heavily marked individuals, the lateral carinal lines are uninterrupted, whereas in less marked individuals, they are interrupted at the intersegmental suture. With regards to venation, the number of cells in the HW anal loop varies from 17- 20, and the extent of Pt from 3–3.5 cells. In females, the colour of dorsal spots on abdomen in S4–S7 varies from deep yellowish-white to bright yellow. The colour of the epiproct may vary from pale yellowish-white to

bright yellow. Tenerals are very different in colour (Fig. 3B–E). In terms of venation, the number of cells in the anal loop ranged from 20 to 25. In males, the ante- and postnodal counts may vary, ranging from Ax 17–19, and Px 9–11 in FW and Ax 13–14 and Px 11–12 in HW and females Ax 16–17, Px 9–11 in FW, and Ax 11–13, Px 10–12 in HW. For a detailed description of teneral females, refer to Das *et al.* (2013).

Etymology. The species is named after Dr. Abraham Samuel, a pioneer of odonatology from Kerala, for his contributions to the field and his constant encouragement to the authors. The specific epithet is a noun in the genitive case.

Diagnosis. Males of *Lyriothemis abrahami* **sp. nov.** are identified by the combination of the following characters: two short oval paradorsal streaks on the mesepisternum not extending halfway up the thorax towards the alar sinus; anterolateral yellow thoracic band as broad as or narrower than the black area just posterior to it; in anteroventral view hamule trapezoid, higher than wide; hamular inferolateral angle right-angled and well-defined, medial border with a distinct insinuation at the junction of its inferior and middle third, inferior border straight. The elongated shape of the hamule is a distinct characteristic of the new taxon. Sexual dimorphism is strongly exhibited in *L. abrahami* **sp. nov.**, with the females identified by their jet-black and yellow colour with characteristic triangular yellow streaks on the dorsum of the abdomen and the distinct triangular shape of the vulvar lamina. The new species is endemic to the Western Ghats of Peninsular India, south of Coorg, and its distribution does not overlap with the range of *L. flava,* as far as it is known.

Key to the *Lyriothemis* males of the Indian subcontinent

(excluding Andaman and Nicobar Is., based on Fraser, 1936 and van der Poorten, 2009)

1. Abdomen black, with S2–S5 pruinosed bluish white.....*L. mortoni* Ris, 1919

– Abdomen blood-red, more or less marked with black.....2

2. Nervures Riii, IRiii, Riv+v and MA abruptly curved downwards and then slightly bent basad at their ends.....*L. cleis* Brauer, 1868

– Nervures Riii, IRiii, Riv+v and MA gently curved at their ends.....3

3. Large species with abdomen 30 mm or more in length.....4

– Smaller species with abdomen <30 mm ……..……..…...5

4. Mesepisternum without yellow streaks; inner margin of hamulus distinctly clefted or incised.....*L. bivittata* (Rambur, 1842)

– Mesepisternum with distinct yellow streaks; inner margin of hamulus not clefted or incised, instead may be shallowly insinuated or straight.....6

5. Small species (AL<20 mm); antehumeral stripes broad above, narrow below, and sides with yellow largely obscured.....*L. acigastra* (Selys, 1878)

– Medium-sized species (AL 26 mm); antehumeral stripes broad all along; sides with yellow stripes and spots.....*L. defonsekai* van der Poorten, 2009

6. Paradorsal streaks on mesepisternum long, extending more than half the way up the synthorax towards the alar sinus; anterolateral yellow thoracic band broader than the black area just posterior to it; in anteroventral view hamule rectangular, broader than high, depressed in the centre, inferolateral angle rounded, medial, inferior border straight.....
....*L. flava* Oguma, 1915 (= *L. tricolor* Ris, 1916)

– Paradorsal streaks on mesepisternum short, extending halfway or less up the synthorax towards the alar sinus; anterolateral yellow synthoracic band as broad as or narrower than the black area just posterior to it; in anteroventral view hamule pentagonal, higher than broad, not depressed in the centre, hamular inferolateral corner right-angled and well-defined, medial border with a distinct insinuation at junction of its inferior and middle third, inferior border straight..... *L.*

abrahami Sadasivan, Jose, Nair & Palot, **sp. nov.**

Distribution: The new species is rare and inhabits semi-evergreen forests, moist deciduous forests, West-coast Evergreen forests, Myristica Swamps, and Subtropical Evergreen forests at elevations of 50–1000 m ASL in Kerala and Karnataka in the southern and central Western Ghats of Peninsular India (Fig. 1). The flight period, based on our observations and fieldwork spanning the last 12 years, is April to December. The regional site records are Kallar Valley (200m ASL), Ponmudi Hills (800–1000m ASL), and Neyyar WLS (500m ASL), in Thiruvananthapuram District, Kerala, and Shendurney Wildlife Sanctuary (50m ASL), Kollam District, Kerala, from Agasthyamalai landscape; Edamalayar-Pooyamkutty Valley (10m ASL) from Lower Periyar landscape; Nilambur (150m ASL), Malappuram District, Kerala, and Silent Valley (900–1000m ASL), Palakkad District, Kerala, in Nilgiri landscape; Periya (550m ASL), Wayanad District, in Wayanad landscape; Aaralam Wildlife Sanctuary (50m ASL), Kannur District, Kerala (Palot and Kiran 2016), and Kakkabe near Madikeri (1100m ASL), Coorg District, Karnataka (KS, per. obs.), in Coorg landscape.

Note on behaviour: In India, *Lyriothemis flava* and *L. bivittata* are reported from heavy jungles near marshes (Fraser 1936). Males are encountered perched on vegetation near phytotelmata, guarding tree holes. They fly when disturbed and return to the same or nearby perch. Females oviposited in the afternoons (Fig. 3F), while males were seen perched and guarding the tree holes (Fig. 3A). We have successfully raised larvae of *L. abrahami* **sp. nov.** collected from phytotelmata and studied their behaviour and ecology since 2022. Larvae of *L. cleis*, *L. magnificata*, and *L. flava* have been reported from phytotelmata (Orr 2003, 2005; Lieftinck *et al.*, 1984). The finer details, including results of phylogenetic analysis, larval morphology, and ecology, will be published elsewhere (in prep.).

DISCUSSION

Notes on previous records of *'Lyriothemis tricolour'* from WG:

The range extension of *L. flava* to the WG was first reported by Das *et al.* (2013) as *L. tricolor* based on the morphology of the larvae, exuvia, and teneral female. The description of the female in Das *et al.* (2013) was based on a teneral specimen, with identification following Fraser (1936). Based on our study of female teneral specimen ZSI/WGRC/IR-INV-2659 used in Das *et al.* (2013) available in ZSI Kozhikode, we confirm that the species mentioned in Das *et al.* (2013) is indeed *L. abrahami* **sp. nov.**, and the lack of male specimens for examination probably led Das *et al.* (2013) to conclude it was *L. tricolor,* even though the larvae they described had some morphological differences from *L. tricolor*. The mature adult female is a very different insect, predominantly black and marked with yellow, compared to males. The new species is sexually dimorphic, with females lacking reddish-orange on the abdomen.

The species groups of *Lyriothemis* of the Western Ghats:

Four species groups were assigned by Ris (1919). Group 1 is characterised by narrow wings, a short and blunt anal loop, many cubital nervures in both wings, and elongated hamules extending horizontally. Group 2 has broader hindwings, longer anal loops, fewer cubital nervures in the forewings (only two), and a discoidal field in the forewings that is wide at the margin. The hamules are similar to those of the next group, but the anterior lamina is distinct. Group 3 is characterised by broad hindwings, a well-developed anal loop, curved main veins (Riii, IRiii Riv+v, and MA), and broader, bowl-shaped hamules. Group 4, the Sino-Japanese group, has broad hindwings, a well-developed anal loop, few cubital nervures, main veins that are not recurved, longer genital lobes, and longer, more angular hamules with a prominent inner yellow membrane. van der Poorten (2009) stated that *L. flava*, *L. cleis,* and *L. bivittata* belong to Group 3, while *L. mortoni*, *L. defonsekai* and *L. acigastra* are assigned to Group 4. *Lyriothemis abrahami* **sp. nov.** fits into Group 3 with its broad hindwings, well-developed anal loop, curved main veins (Riii, IRiii, Riv+v, and MA), and broad hamules.

The description of *L. abrahami* **sp. nov.** brings

Fig. 3 *Lyriothemis abrahami* **sp. nov.** in life. A–mature male from Shendurney Wildlife Sanctuary © Kalesh Sadasivan; B–lateral view of a teneral male © Jebine Jose; C–dorsal view of a teneral male © Jebine Jose; D–lateral view of a teneral female © Jebine Jose; E–dorsal view of a teneral female © Jebine Jose; F–female ovipositing in a phytotelma © Jebine Jose. (B–E, reared from the larvae collected in Pooyamkutty, Ernakulam District, Kerala)

the total number of odonates in Kerala to 191, with 78 endemics. With its wide distribution across all landscapes and elevations, examination of museum specimens, it is concluded that the historical records of *L. flava* (= *L. tricolor*) from Kerala and the WG, are of *L. abrahami* **sp. nov.** The discovery of *L. abrahami* **sp. nov.**, which was misidentified in previous studies from the region because of its superficial resemblance to *L. flava*, reiterates the need for careful study of the morphology of odonates, which may be further supported by

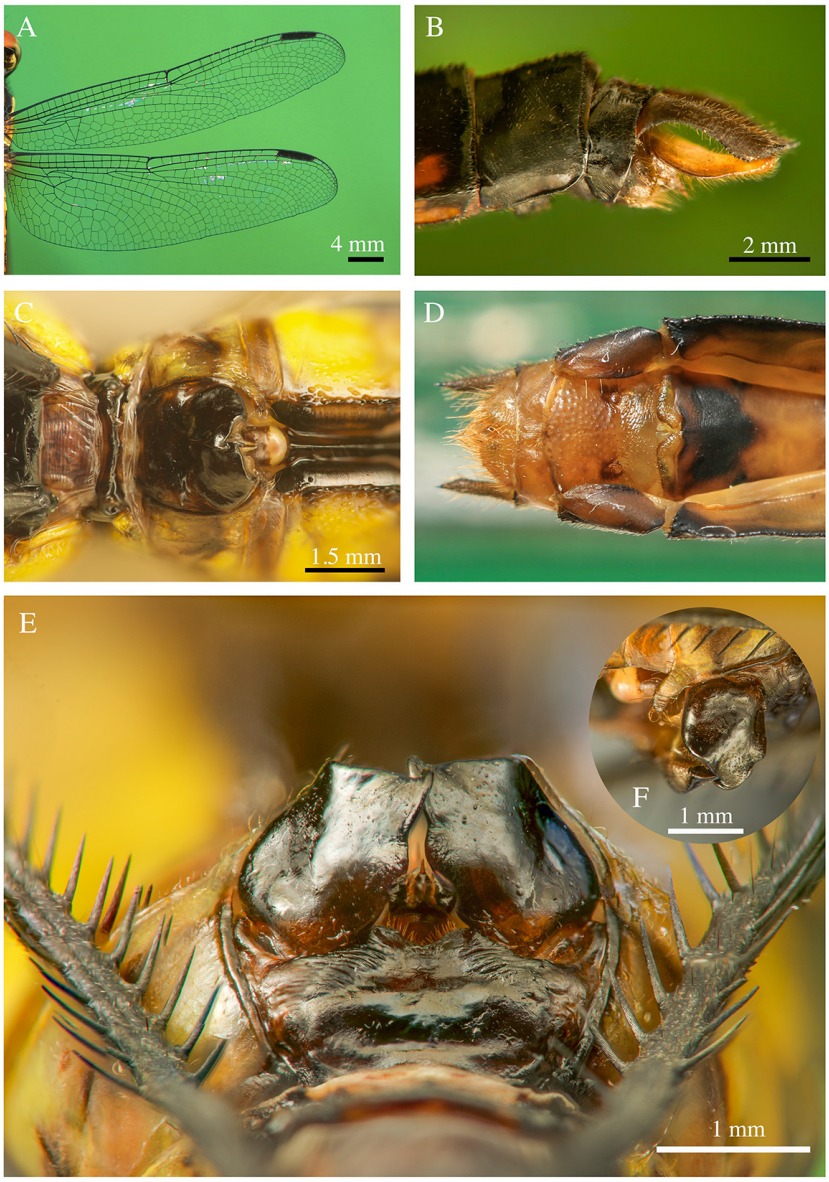

Fig. 4 *Lyriothemis abrahami* **sp. nov.** A–venation of male TORG # 1053 © Kalesh Sadasivan; B–lateral view of male S10 and appendages TORG # 1053 © Kalesh Sadasivan; C–genital fossa of male TORG # 1053 © Kalesh Sadasivan; D–ventral view of female terminal segments showing the vulvar scale © Jebine Jose.; E–inverted anteroventral view of male hamules © Kalesh Sadasivan; F–lateral view of male hamule TORG # 1053 © Kalesh Sadasivan

phylogenetic and larval studies. An integrated approach to taxonomy is needed for the careful species delimitation and description of odonates.

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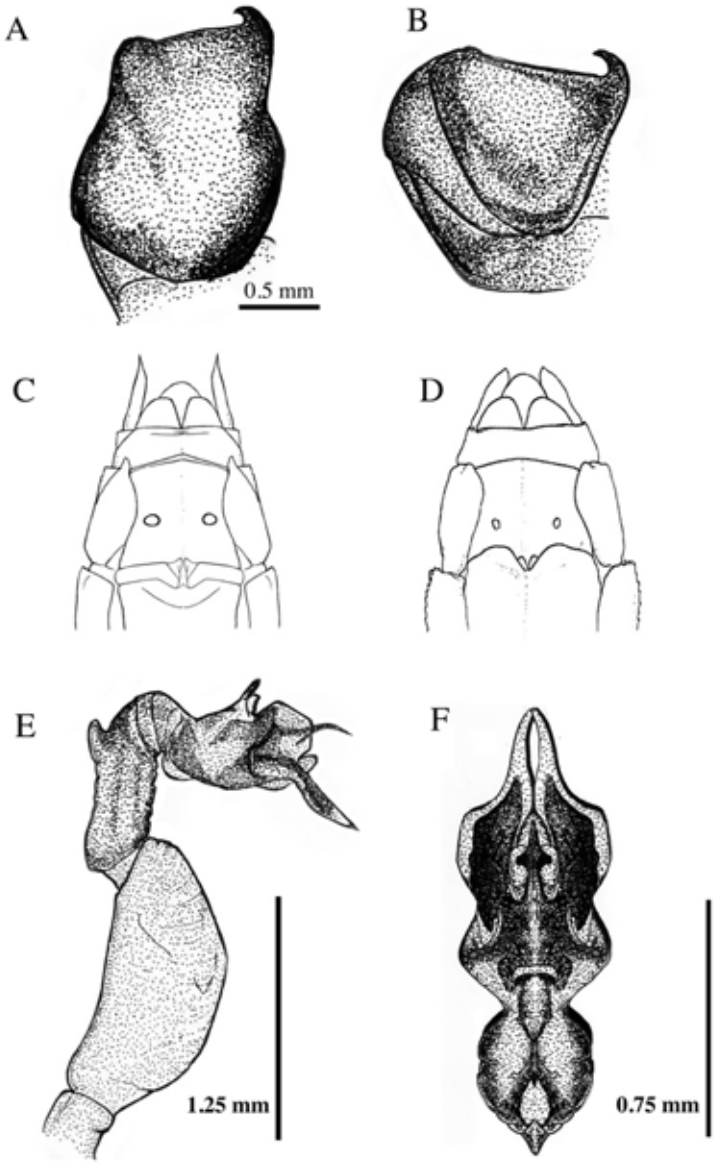

Fig. 5 Comparative illustrations of male and female genitalia of *Lyriothemis abrahami* **sp. nov.** (A, C, E) and *L. flava* (B, D, F). A–hamules of *L. abrahami* **sp. nov.** based on holotype TORG # 1053; B–hamules of *L. flava* redrawn from Kompier (2017) (not to scale); C–vulvar scales of female *L. abrahami* **sp. nov.**; D–vulvar scales of female *L. flava* Oguma, 1915 redrawn from Ozono *et al*. (2021); E–lateral view of vesica spermalis of *L. abrahami* **sp. nov.**; F–dorsal view of segments 3 & 4 of vesica spermalis of *L. abrahami* **sp. nov.** © Kalesh Sadasivan

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An unusual new record of *Pseudoathyreus orientalis* (Laporte, 1840) (Coleoptera, Bolboceratidae, Athyreinae), an eremian species from the Deccan Peninsula, India and designation of its neotype

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ABSTRACT: *Pseudoathyreus orientalis* (Laporte, 1840) is newly reported from Deccan Peninsula. It is an unusual record of the earth-boring beetle from Gautala-Autramghat Wildlife Sanctuary (GAWLS), Jalgaon, Maharashtra, India. The description of the female adult with images and distribution map of the *P. orientalis* are provided. In an effort to stabilize the specific epithet and to fix the taxonomic status of this rare species, a neotype is designated. © 2025 Association for Advancement of Entomology

KEY WORDS: Distribution, light trap, earth-boring beetle

The tribe Athyreini is a small and lesser-known tribe of Bolboceratidae consisting of four genera (*Athyreus, Pseudoathyreus, Parathyreus*, and *Neoathyreus*) (Carpaneto *et al*., 2000; Bai *et al*., 2017). This tribe is less studied due to the fact that they are not attracted to the light frequently (Bai *et al*., 2017). In recent past a few new species have been discovered (Boilly, 2014; Carpaneto and Romiti, 2020; Ferreira *et al.,* 2023; Celante *et al.,* 2024), but still studies are not sufficient on the diversity and ecology on this tribe. The first fossil beetle of the tribe Athyreini is described in 2016 by Bai *et al*. (2017) from Myanmar.

The Afro-Indian genus *Pseudoathyreus* Howden and Martínez, 1963 is known from Africa and Asia with ten extant species (Poggi *et al.,* 2019; Carpaneto *et al*., 2000; Sommer *et al*., 2020; Schoolmeesters, 2025). There are ten valid species namely Afrotropical -*Pseudoathyreus damara* (Kolbe, 1907), *P. flavohirtus* (Walker, 1871), *P. fracticollis* (Fairmaire, 1882), *P. freyi* (Gomes Alves, 1957), *P. kordofanus* (Klug, 1843), *P. porcatus* (Castelnau, 1840), *P. rhodesianus* (Péringuey, 1908) and Oriental – *P. orientalis* (Castelnau, 1840). Here, *P. frontalis* (Parry, 1845), a second Asian species is not considered as valid (Carpaneto and Romiti, 2020). Carpaneto and Romiti (2020) has given two hypotheses on the validation of *P. frontalis*. In their words "*Pseudoathyreus frontalis* was described from NE India (Khasi Hills, Meghalaya) but the type material of this species is completely unknown to the curators of all the museums contacted and the original description (Parry, 1845) is very basic and scarcely detailed, and led us to suggest that this species could either be a junior synonym of *P. orientalis* or belong to another genus of

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bolboceratid beetle. The last hypothesis is more probable because the type locality falls within a moist tropical forest area whereas all other known distribution records for this species and for the other *Pseudoathyreus* species fall within drier habitats (at most arid savannas or semideserts)". For more details see Carpaneto and Romiti (2020). Thus, *P. orientalis* is the only valid species from Asia (Kral and Batelka 2017; Carpaneto and Romiti 2020).

An eremian beetle, *P. orientalis* is distributed in India and Pakistan (Carpaneto and Romiti, 2020; Sommer *et al*., 2020). It is described from "Bengale" by Laporte in 1840 as *Athyreus orientalis*. During that time, Bengale was a part of the former Bengal Presidency, which included Bangladesh, fell under British India. The type of it seems to be untraceable (Carpaneto and Romiti 2020). Further, Carpaneto and Romiti (2020) reported that the non-type specimens of *P. orientalis* are kept either in the Natural History Museum of London (NHM) or in the Museum National d'Histoire Naturelle of Paris (MNHN). While searching the database of both the museum, the author found database of the non-type specimens housed in NHM, London (Natural History Museum 2014). In compliance with Article 74 of the International Code of Zoological Nomenclature (ICZN, 1999), a neotype for *P. orientalis* is designated in this study. This was done to stabilise the nomenclature by selecting a single specimen as the name bearing type.

It is a species of dry habitats (deserts, semideserts or moist arid savannas) and supposed to be a desert species. But, during this study a single female specimen of *P. orientalis* was collected from GAWLS, Jalgaon, located in the north-west region of the state of Maharashtra, India. The report of this species from GAWLS forms the first record of *P. orinetalis* from Deccan Peninsula part of Maharashtra. The vegetation of this sanctuary is classified as dry deciduous forest ecoregion of Central Deccan Plateau characterized with extreme hot summers. The main river here is the Tapti, which unlike other rivers in the state that flow eastward towards the Bay of Bengal, flows westward from its headwaters in eastern Maharashtra to empty into the Arabian Sea. In Jalgaon District, the average annual rainfall is around 690mm and the temperature ranges from 10 to 48 ^{o}C, making it a diverse climate with scorching summers of up to 45 ^{o}C. GAWLS lies in the Satmala and Ajanta hill ranges of the Western Ghats. The sanctuary does not fall under the desert ecoregion, but has extreme hot summers with less rainfall and semi-arid climate. Since, GAWLS does not fall under desert eco-region, this discovery is an unusual record. As mentioned earlier, studies on this group is less and so there are chances of many more range extension record of this species from India.

The specimen was collected using a light trap installed in the sanctuary in the night. The collected specimen was killed with ethyl acetate vapours. Further, it was relaxed, pinned, and dry preserved in the laboratory. The identification of the specimen was done with the help of Král and Batelka (2017) and Sommer *et al*. (2020). The specimen was studied under a Leica S9i stereozoom microscope with photographic facility. The images were stacked using Combine ZP software and then processed with Adobe Photoshop CS Version 8. The identified specimen labelled as neotype was duly registered and deposited in the National Zoological Collection, Zoological Survey of India, Western Regional Centre, Pune, Maharashtra, India (ZSI–WRC). The detailed collection locality is given under material examined and also shown in Fig. 1. The map of the collection locality was prepared using the open, free access QGIS software version 3.22.0. The location coordinates have been taken from googlearth for the distribution localities. The collection coordinates for Maharashtra have been recorded using Garmin GPS in the field.

Taxonomy

Insecta: Scarabaeoidea;

Family Bolboceratidae Mulsant, 1842

Subfamily Athyreinae Lynch-Arribálzaga 1878

Tribe Athyerini Lynch-Arribálzaga, 1878

Genus *Pseudoathyreus* Howden and Martínez, 1963

Pseudoathyreus orientalis (Laporte, 1840) (Figs. 1, 2)

Athyreus orientalis Laporte, 1840. *Hist. Nat. Ins. Col*.: 103. Neotype (female), here designated, deposited at ZSI-WRC (Fig. 2).

Type locality: "Bengale" (Erstwhile Bengal Presidency and present-day West Bengal and Bangladesh).

Fig. 1 Map showing distribution of *Pseudoathyreus orientalis* (Laporte, 1840)

Fig. 2 Adult habitus of *Pseudoathyreus orientalis* (Laporte, 1840): A, Dorsal view, B, Lateral view

Diagnosis: Female (Fig. 2A–C): Length: 13mm, width: 8mm. Body convex, brown, shiny dorsally. Fronto-clypeal carina elevated with two lateral teeth. Eye-canthus rectangular with raised portion. Pronotal margins raised covered with thick, long setae. The margin between lateral fossae gradually elevated to small, tubercle on midline. Anteriorly pronotum with concavity sloping, with median longitudinal groove. Inner carina interrupted at middle. Two separate aligned fragments forming outer carina. Underside of the body covered with yellow long and dense setae.

Material examined: One female, with "Neotype ♀" Bhaskaracharya Forest House, Patnadevi, Chalisgaon, Jalgaon (20.318° N 74.975° E), 24.09.2019, P.S. Bhatnagar lgt. (ZSI-WRC-Ent-1/4954).

Distribution: India (Haryana: Kurukshetra (Mittal, 1981; Ghosh *et al*., 2020), Maharashtra (new record reported in the present study), Rajasthan (Frey, 1956), West Bengal (Laporte, 1840; Carpaneto *et al*., 2000; Král and Batelka, 2017)) and Pakistan (Sommer *et al*., 2020).

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First record of the genus *Lemuriana* Distant, 1905 (Hemiptera, Cicadidae, Lamotialnini) from Kerala, India

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ABSTRACT: The cicada *Lemuriana apicalis* (Germar, 1830) (Hemiptera, Cicadidae, Lamotialnini) is reported for the first time from Kerala, India. © 2025 Association for Advancement of Entomology

KEY WORDS: Range extension, cicada, distribution, *Lemuriana apicalis*, Western Ghats

Cicadidae, one of the two families of cicada, comprises slightly more than 3000 species and encompasses practically all extant cicada species in the world (Zhou and Lei, 1997; Sanborn, 2013; Marshall *et al*., 2016, 2018; Barlett *et al*., 2018; Simon *et al*., 2019). The genus *Lemuriana* Distant, 1905 belongs to the tribe Lamotialnini in the family Cicadidae and subfamily Cicadettinae (Marshall *et al*., 2018). The genus *Lemuriana* was erected by Distant for *L. apicalis* (Germar, 1830) from Bengal and *L. flavocostata* Distant, 1905 from British East Africa (Distant, 1905). *Lemuriana apicalis* was designated as the type species of the genus (Distant, 1906b). The African species of the genus *Lemuriana– L. flavocostata* Distant, 1905, *L. consobrina* Distant, 1920 and *L. sirius* Distant, 1899 were transferred to the genus *Trismarcha* Karsch, 1891 by Boulard in 1975. *Lemuriana connexa* Distant, 1910 from Borneo was transferred to the new genus *Unduncus* Duffels, 2011 and *L. chandaea* Moulton, 1911 from Borneo was made a junior synonym of *Abroma kedenburgi* and transferred to the new genus *Allobroma* Duffels, 2011. In its current definition *Lemuriana* includes three species: *L. apicalis* (Germar, 1830) from Bengal and India, *L. cambodiana* Lee, 2010 from Cambodia (Lee, 2010) and *L. vinhcuuensis* Pham and Yang, 2010 from Vietnam (Pham and Yang, 2010).

During field work for cicadas in a dry deciduous forest in the Attapadi region of Palakkad district of Kerala state, in the Nilgiris landscape, the authors came across a few cicadas that matched the description of *Lemuriana*. These specimens were collected using long handled insect collecting nets, preserved in absolute alcohol and pinned for study. Detailed morphological examination was done with HEADZ Model HD81 stereomicroscope. Photographs were taken with Nikon D90 Digital SLR camera and 50mm f/1.8 Nikkor lens. (Figure 2 &3). The pinned and dried specimen was taken and the last sternite was removed to expose the pygofer of the male genitalia. Pygofer in ventral

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and lateral view examined using HEADZ Model HD81 stereomicroscope and photographed with Canon 70D Digital SLR camera, Canon 180 mm macro lens, and MPE 65 f 2.8 1e5x Lens (Fig. 4). Morphometric measurements of the adult cicadas were obtained from images using ImageJ (64-bit Java 1.6.0) software. For each of the two specimens, each morphometric parameter was measured five times, resulting in a total of 10 measurements per parameter. The mean and standard deviation were subsequently determined for each of the morphometric parameters. Finally, the standard error is calculated by dividing the standard deviation by the square root of the number of measurements. The indices and measurements used in the description are in cephalocaudal order, as per Sadasivan (2021), and are taken in millimetres (mm) in the dorsal view unless otherwise specified. Head length (HL)—length of the head in the midline from the anterior- most point of the postclypeus to the mid-posterior margin of the head, measured dorsally. Head width (HW)—width of the head including the compound eye, measured between the lateral-most points of convexity of the compound eye in dorsal view in the transverse plane. Eye length (EL)—in the dorsal view. Pronotum length (PL)—at the mid-dorsal line. Pronotum width (PW)—maximum width, measured in dorsal view. Mesonotum length (ML)— Mesonotum mid-dorsal length to the cruciform elevation, in dorsal view. Mesonotal width (MW)— width of Mesonotum. Forewing length (FWL)—the maximum expanse of the forewing from its medial most attachment to the mesonotum to the most convex part of its apex. Forewing width (FWW)—distance between the node and the tornus across the forewing. Hindwing length (HWL)—the maximum expanse of the hindwing from its medial most attachment to the mesonotum to the most convex part of its apex. Abdomen length (AL)—mid- dorsal length of the abdomen measured from the posterior-most point on the cruciform elevation to the tip of the pygofer or anal style, whichever is the farthest, in the freshly killed insect. Abdomen width (AW)—the maximum width measured in the transverse plane in dorsal view. Operculum length (OPL)—in lateral view. Rostrum length (RL). Anterior body length (ABL)—length of the specimen from the anterior tip of postclypeus to the posterior of scutellum in the midline. HL + PL + ML is Total Length (TL); HL + PL + ML + AL is Cephalic Index (CI); (HW/HL) × 100 is Ocular Index (OI); (EL/HW) x 100 is Pronotal Index (PI); (PW/PL) × 100 is Mesonotal Index (MI); (MW/ML) × 100 is Opercular Index (OPI); (OPL/ABL) × 100 is Anteroposterior Index (API); ABL/AL × 100 is Rostral Index (RI); (RL/ABL) × 100 is Forewing Aspect Ratio (FAR), high aspect ratio indicates long, narrow wings, and a low aspect ratio indicates short, wide wings. (FWL/FWW) × 100 is Forewing Index (FI); (FWL/ABL) × 100 is Inter-Wing Ratio (IWR); (FWL/HWL) × 100 is Gastral Index (GI); AW/AL x 100, high index value indicates a relatively wider abdomen. The diagnosis and brief description of *L. apicalis* along with morphometric measurements are provided below for the specimens from Kerala.

Family **Cicadidae Batsch, 1789;**
Subfamily **Cicadettinae Buckton, 1890**

Tribe **Lamotialnini Boulard, 1976**
Genus ***Lemuriana*** **Distant, 1905**

Type species ***Lemuriana apicalis***
(Germar, 1830)

Diagnosis: *Lemuriana apicalis* was formerly classified under the tribe Taphurini based on morphological characteristics (Duffels, 2011). However, Marshall *et al.* (2018) transferred Lemuriana to Lamotialnini based on molecular phylogenetic analysis, which demonstrated a closer evolutionary link with other genera in Lamotialnini. This molecular evidence, which studied evolutionary divergence and genetic clustering, formed the basis for its reclassification.

Short description: Head of *L. apicalis*: Notable postclypeus and vertex; with ferruginous markings. Pronotum: Broad black median fascia, expanded ends; middle portion obliterated; lateral portion black. Mesonotum: Black with paramedian yellow-brown fasciae, narrow anteriorly, widening along parapsidal sutures, narrowing near cruciform elevation; para median and lateral sigillae dark reddish-brown to black. Tegmen: Apical area lightly infuscated (dark/light brown); venation dark yellow

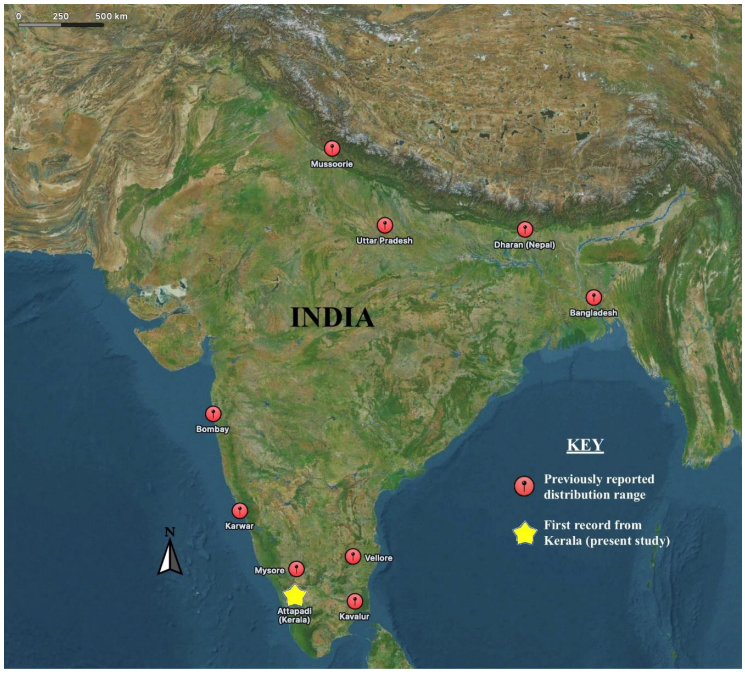

Fig. 1 A map showing the distribution of *Lemuriana apicalis* (Duffels, 2011 and Price *et al*. 2016). Modified from Apple maps, Copyright © 2012–2020 Apple Inc.

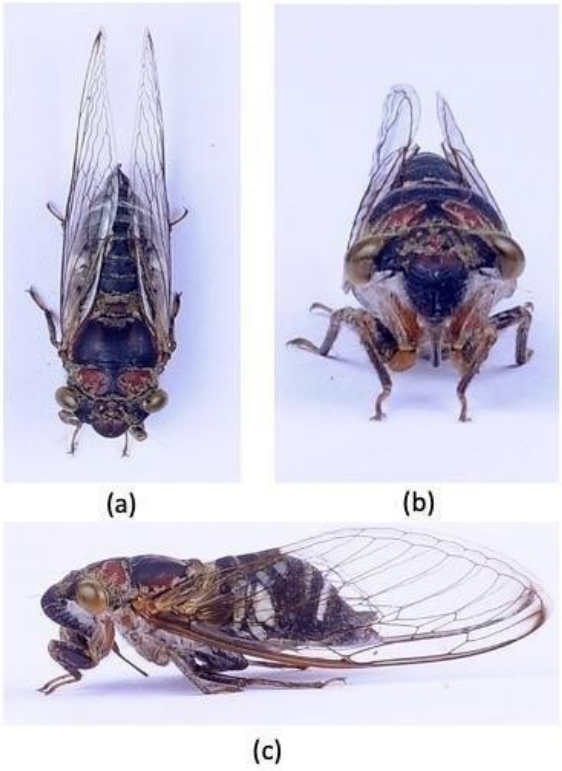

Fig. 2 *Lemurina apicalis* **(a)** Dorsal view of the whole insect **(b)** Anterior view of the head. **(c)** Lateral view of the whole insect ©Anjana A.

Fig. 3 *Lemuriana apicalis*, male, dorsal view ©Anjana A.

to brown. Wings: Light brown, suffused on distal anal lobe. Legs: Light brown. Male operculum: Brown to black, extending to or beyond tympanal cavity; medial margin straight; lateral margin sinuate, concave basally, convex distally (Duffels, 2011).

Material Examined: 2♂♂; 04-vi-2024; Attapadi, Palakkad District, Kerala, India, (11º5' 23.64'' N; 76º 37' 21.72'' E); Dry deciduous forests; 957m ASL; Coll. Anjana. A.

Measurements (n=2): FWL–23.454±0.265;

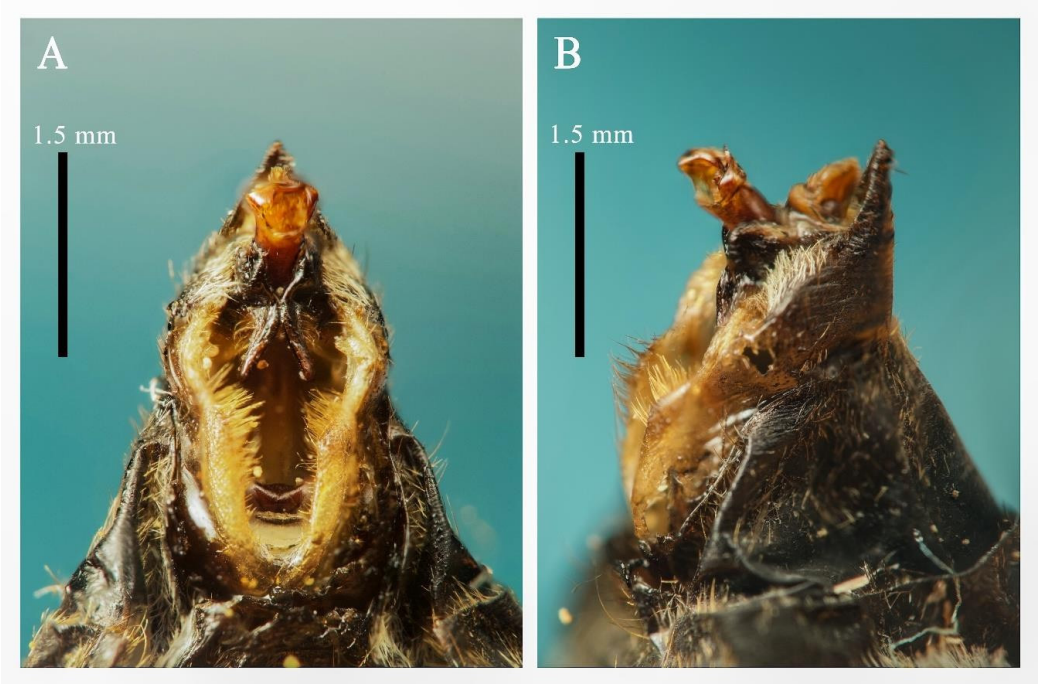

Fig. 4 *Lemuriana apicalis*, male, (A) Pygofer in ventral view (B) Pygofer in lateral view ©Kalesh Sadasivan

FWW–7.99±0.055; HWL– 11.872±0.091; HL–2.247±0.038; HW–6.92±0.118; EL–1.774±0.054; PL–2.259±0.056; PW–5.825±0.024; ML–4.305±0.125; MW–6.224±0.2; AL 9.863±0.037; AW–5.719±0.263; OPL–5.044±0.213; RL 1.787 ±0.197; ABL–8.811±0.142; TL–18.674± 0.147; CI 307.966±.7.396; OI–25.636±0.894; PI–257.857 ±6.48; MI 144.576±6.261; OPI–57.247± 2.588; API– 89.334±1.478; RI–20.281±2.26; FAR–293.542±3.884; FI–266.19±5.239; IWR–197.557± 2.697; GI–57.984±2.675.

Updated Distribution: Attapadi (Palakkad district, Kerala); Bombay, Karwar, Bengal, Northern India, Mysore, United Provinces, Central Provinces (Price *et al.,* 2016); Oriental Region (Distant, 1906a, b); Mussoorie (Metcalf, 1963); Uttar Pradesh (Duffels and van der Laan, 1985) in India; Indochina & Nepal (Sanborn, 2014); Bangladesh (Duffels, 2011), and in Vietnam (Pham and Yang, 2010).

Remarks: In its present concept the genus *Lemuriana* encompasses three species: *L. apicalis* from Bombay, Karwar, Bengal, Northern India, Mysore, United Provinces, Central Provinces (Price *et al.,* 2016); Oriental Region (Distant, 1906a, b); Mussoorie (Metcalf, 1963); Uttar Pradesh (Duffels and van der Laan, 1985) in India; Indochina & Nepal (Sanborn, 2014); Bangladesh (Duffels, 2011), Vietnam (Pham and Yang, 2010) and Attapadi, Kerala (present study), *L. cambodiana* Lee, 2010 from Cambodia (Lee, 2010) and *L. vinhcuuensis* Pham and Yang, 2010 from Vietnam (Pham and Yang, 2010). The detailed description of the three species in the genus is diagnosed and compared as per Lee (2010), Pham and Yang (2010) and Duffels (2011). The specimens exhibited a rugged head margin characterized by a prominent postclypeus and vertex, an apical fuscous spot on the forewing, an ochraceous costa, a fuscous operculum, and large lateral white spots extending to tergite 2, which are distinctive features of *L. apicalis*. Conversely, the other two species do not possess the apical spot, exhibit smoother head margins, and display variations in abdominal markings. The male genitalia, characterized by a slender, curved aedeagus and tapering uncus, aligns with *L. apicalis,* effectively excluding other species (Lee, 2010; Pham and Yang,

2010; Duffels, 2011). Hence, the specimens we examined from Kerala are identified and confirmed as *L. apicalis* (Germar, 1830). However, cicadas of the region need to be studied more thoroughly, particularly with emphasis on their distribution and ecological roles.

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Evaluation of food consumption of *Zygogramma bicolorata* Pallister 1953, on *Parthenium hysterophorus* in Dehradun, Uttarakhand, India

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ABSTRACT: The feeding potential and non-feeding potential of Mexican beetle, *Zygogramma bicolorata* Pallister, 1953 (Coleoptera, Chrysomelidae) were studied on a common weed *Parthenium hysterophorus,* under laboratory conditions (temperature 25 to 30 ^{0}C and RH 60 to 65%). Ten different sites were chosen from the Dehradun district considering the widespread availability of Parthenium weed in the region. It was observed that the feeding potential of the adult was highest in site 7 (Prem nagar). The feeding rates at the site 7 were, 0±0.00 at 0 hours, 1.8±0.11 at 24 hours, 2.77±0.14 at 48 hours, 3.87±0.03 at 72 hours, 2.74±0.11 at 96 hours, 2.6±0.05 at 120 hours, 0±0.00 at 144 hours and at 2.67±0.07 at 168 hours. *Z. bicolorata* was more active when it was used in higher density and at initial growing stages of the weed. The fully matured adult stages of *Z. bicolorata* were less potential in comparison to the younger ones. Non-feeding potential was tested on different weeds and grasses indicated that the beetles were exclusive feeders of Parthenium weed and contributes towards achieving the goal of sustainable development.
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KEY WORDS: Feeding potential, defoliation, non-feeding potential, behaviour

Parthenium hysterophorus is a troublesome annual unwanted weed in agricultural fields, grazing lands and fallows in India. India has joined the list of heavily affected by *P. hysterophorus* nations in the world as this weed has widely spread in most of the states of the country in both cultivable, barren and pasture lands (Vartak, 1968). The weed *P. hysterophorous* is from the family Asteraceae is an international weed and is one of the worst weeds found in the world. This weed is a 1-2m tall herb with simple, grey-green, deeply lobed and hairy alternate leaves. It bears cream-colored flowers about 4mm in diameter at the tip of each branch and its seeds germinate profusely after rain (Aneja *et al.,* 1991; Uniyal *et al.,* 2001). This reduces forage production in grasslands by 70 percent and makes the land less fertile (Vartak, 1968). This weed has been reported as a serious health risk, especially for agricultural workers. Since then, the weed has become a menace in various parts of the country, various methods have been used for its eradication such as manual removal of plants (Joshi 1990), use of herbicides cultural practices such as intercropping

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and breeding of competing plants such as forage sorghum or maize (Joshi, 1990), self-seeding species such as *Cassia sericea* SW (Joshi and Mahadevappa, 1986) and biological control using insects is recommended to its control. *Zygogramma bicolorata* Pallister, 1953 (Coleoptera, Chrysomelidae), feed on *P. hysterophorus* leaves and flowers. Beetles are creamish brown in colour with dark brown and black longitudinal markings on the elytra, measuring about 6 to 7mm in length. They lay elongated oval yellow-colored eggs on the ventral side of the leaves. It is followed by the larval stage which includes first instar, second instar, third instar and fourth instar stage. The larval stages feeds for around two weeks and then pupate into the soil. New adults appear after almost 15 to 20 days. Single beetle is able to complete its cycle in around 30 to 40 days depending on the environmental conditions. The insect can have up to 6 generations in the field. The female insect has the capacity of laying 2000 to 3000 eggs in its lifetime. *P. hysterphorus* has been found to cause serious health problems in humans and animals (Sushilkumar, 2005). The feeding potential and non-feeding potential of the beetle were undertaken on *Parthenium hysterophorus,* in ten different sites chosen from the Dehradun district considering the widespread availability of Parthenium weed in the region.

The study area is surrounded by the Himalayan ranges on the outer sides and Shivalik hills. The deposits of the Doon Valley, having their origin Himalayan comprise of heavy boulders of gravels, coated with thin covering of soil, which shows frequent outcrops of its rocky subsoil. The city is situated in the middle of the valley at the altitude of 636m. Ten study sites are selected in Dehradun region on the basis of survey of beetle in the surrounding areas. The sites are site 1 (Garhi Cantt), site 2 (Kaulagarh), site 3 (Raipur), site 4 (Rajpur), site 5 (Doiwala), site 6 (Sahastradhara), site 7 (Prem Nagar), site 8 (Jamunwala), site 9 (Anarwala) and site 10 (Jhajra).

The adult beetles collected from parthenium leaves and flowers were reared from December to May in the laboratory under simulation conditions (27±5 °C and RH 35±5 %), in the plastic jars. For selection of plant feeding study bowl (500 ml) were used. Temperature (25 to 30 °C) and RH (60 to 65%) were maintained using desert cooler in the laboratory at Shri Guru Ram Rai University. The experiment conducted in laboratory on the basis of Jaiswal *et al.* (2023) with minor modifications.

Choice test: For the experiment 5 beetles were placed in each study bowl with 500ml capacity with 5g of parthenium leaves and each test replicated three times (n=3) with the control. Weighing scale is used to take accurate and same quantity of Parthenium in every bowl. For the choice test parthenium leaves was placed after 0, 24, 48, 72, 96, 120, 144 and 168 hour's total weight of parthenium leaves was measured 5g Parthenium weight is considered as its control weight for the experiment. Food was changed every third day.

Non-choice test: For the experiment 5 beetles were placed in each study bowl with 500ml capacity with 5g of other weed and grasses (*Cynodon dactylon* and *Lantana camara*) and each test replicated three times (n=3) with the control. Weighing scale is used to take accurate and same quantity of grasses in every bowl. For the non-choice test grass leaves was placed after 0, 24, 48, 72, 96, 120, 144 and 168 hour's total weight of grass leaves was measured. Five gram grass weight is considered as its control weight for the experiment. Food was changed every third day.

Effect of feeding rate = Control Weight of leaves– Weight of leaves feeding by beetles

In the choice test maximum consumption of *P. hysterophorus* was occurred in site 7 (Prem nagar) and minimum in site 2 (Kaulagarh). The feeding consumption ranged from 1.8±0.05 to 1±0.11, maximum at site 7 (1.8±0.11), followed by site 10 (1.74±0.03) and minimum at site 1 (1±0.11) at 24 hours. At 48 hours, food consumption ranged from 2.77±0.14 to 2.24±0.12, most at site 7 (2.77±0.14), followed by site 4 (2.74±0.08) and least at site 1 (2.24±0.12). At 72 hours, consumption ranged from 3.87±0.03 to 3.24±0.08, maximum at site 7 (3.87±0.03), followed by 3.77±0.03 at site 10 and least at site 1 (3.24±0.08). At 96 hours, food

Table 1. Consumption of *Z. bicolorata* at different hours on *P. hysterophorus* during Choice Test (Mean±SE)

Site/ hour	24	48	72	96	120	144	168
1-Garhi Cantt	1.00±0.11	2.24±0.12	3.24±0.08	2.54±0.18	3.6±0.05	0.00±0.00	2.3±0.20
2-Kaulagarh	1.54±0.17	2.50±0.11	3.37±0.08	1.17±0.18	2.44±0.12	0.00±0.00	1.9±0.11
3-Raipur	1.44±0.20	2.57±0.00	3.54±0.12	1.64±0.17	2.5±0.12	0.00±0.00	2.3±0.10
4-Rajpur	1.27±0.08	2.74±0.08	3.67±0.08	1.5±0.11	2.7±0.11	0.00±0.00	1.54±0.17
5-Doiwala	1.80±0.05	2.7±0.05	3.64±0.08	1.27±0.08	2.47±0.08	0.00±0.00	1.47±0.08
6-Sahastrdhara	1.50±0.05	2.54±0.12	3.7±0.11	1.54±0.12	3.1±0.15	0.00±0.00	2.2±0.15
7-Premnagar	1.80±0.11	2.77±0.14	3.87±0.03	2.74±0.12	3.6±0.05	0.00±0.00	2.67±0.07
8-Jamunwala	1.64±0.14	2.74±0.14	3.54±0.12	1.47±0.14	2.64±0.08	0.00±0.00	2.47±0.08
9-Anarwala	1.57±0.18	2.67±0.12	3.74±0.03	1.14±0.08	2.67±0.14	0.00±0.00	1.84±0.12
10-Jhajra	1.74±0.03	2.57±0.14	3.77±0.03	1.27±0.08	2.67±0.12	0.00±0.00	2.1±0.05

consumption ranged from 2.74±0.11 to 1.17±0.18, maximum at site 7 (2.74±0.11), followed by site 1 (2.54±0.18) and least at site 9 (1.14±0.08). At 120 hours, the feeding ranged from 3.6±0.05 to 2.44±0.02, with maximum at site 7 (3.6±0.05), followed by site 6 (3.1±0.15) and least at site 2 (2.44±0.02). At 144 hours no food was given so the reading comes to 0±0.00. At 168 hours, food consumption ranged from 2.67±0.07 to 1.47±0.08, maximum eaten at site 7 (2.67±0.07), followed by 2.3±0.2 at site 1 and least at site 5 (1.47±0.08) (Table 1).

In non-choice test, no food was consumed in all the ten sites. Leaves of grasses given in the feeding bowl at the beginning remained unconsumed till the end. At 144 hours, no leaves were placed and at 168 hours also food consumption remained zero. This indicated the host specific nature of *Z. bicolorata*, meaning it only consumed *P. hysterophorus*.

Similar observations were made by Jaiswal *et al.* (2021) who studied the Parthenium consumption rate of *Z. bicolorata* at both adult and grub stage. Food consumption analysis of *Z. bicolorata* was also done by Saha *et al.* (2017), who did similar work and did their analysis over a period of thirty days and analyzed the impact of *Z. bicolorata* on *P. hysterophorus*. Chandravanshi *et al.* (2018) too analyzed the impact of *Z. bicolorata* and how it can be used in Parthenium management. It was released in the year 1984 and since then, it has spread over an area of more than 200,000km^2 around Bengaluru. Chakravarthy and Bhan (1997) observed the beetle emergence during May at Bengaluru, Tumkur, Hassan and Chikmaglore. Number of adults at each time interval suggested that the beetle underwent four overlapping generations from June to August with an average of almost twenty days for completion of each generation.

This weed has led to a decline in the crop production (nearly 40%) of India (Khosla and Sobti, 1981). Maharjan *et al*. (2007) showed that it acts as an invasive weed hindering the growth of all the plants of the region and deteriorating the soil quality of the area as well. Due to its allelopathic effect it greatly affects the germination rate of crops causing a decline in the productivity as well as natural vegetation of the affected area. Using a bio-control agent is a strategic way of weed management contributing both to the society and environment.

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New distribution of the myrmicine arboreal ant, *Paratopula intermedia* Sheela and Narendran (Formicidae, Myrmicinae) from south India

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ABSTRACT: The myrmicine ant, *Paratopula intermedia* Sheela and Narendran, 1998, which was reported so far only from the type locality (Kerala), was found to be present in two other Indian states as well, Karnataka and Tamil Nadu. © 2025 Association for Advancement of Entomology

KEY WORDS: Occurrence, Tamil Nadu, Karnataka

The arboreal ant, *Paratopula* is a small genus with about 12 species across the world (www.antweb, accessed on 25. 06. 24). Luo and Guénard (2016) updated the identification key for twelve known species of the genus *Paratopula* at global level. The genus is known to be distributed in the Indomalayan region (Luo and Guénard, 2016).

Sheela and Narendran described *P. intermedia* from Muthanga, Kerala in the year 1998 and is known so far only from the type locality, Muthanga, Kerala. Muthanga is apart of Wayanad Wildlife Sacntury situated at a range of elevation of 650 to 110m from sea level. The temperature in the Sanctuary ranges from 13 to 32^{0}C. This place gets annual rainfall of 3000 to 4000 mm and humidity in above 75 per cent even during summer and up to 95 per cent during monsoon. The ecological miche of this partivular ecosystem is well suited for this species. A recent study of the ant fauna of Indian Institute of Science, Bengaluru, revealed the presence of this species in Karnataka. The Indian Institute of Science campus in Bengaluru, Karnataka which is about 900m above sea level and experiences a moderate climate with annual temperature ranging 15 - 26^{0}C and average rainfall 787mm. The humidity on an average is 65.2 per cent and it ranges 45 in March to 79 per cent is August. Another survey at Madurai Kamaraj University also showed the presence of this myrmicine ant in Tamil Nadu. This university is located in Madurai (Tamil Nadu) at an elevation of approximate 176m above sea level. The average anuual temparature is 28.2^{0}C, with an annual average rainfall of 849mm and average hunidity 69.7 per cent.

Thus this myrmicine ant spicis *P. intermedia* is avle to exist in a wide range of ecological conditions, with varied elavation, temparature, rainfall and humidity. Though *P. ceylonica* is reported from Karnataka (Bharti *et al.,* 2016), *P. intermedia* is not reported to be present in either Karnataka or Tamil Nadu.

Paratopula intermedia Sheela and Narendran, 1998

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Fig. 1 *Paratopula intermedia* worker – profile

Fig. 2 *P. intermedia* worker, head, frontal view

Diagnostic features: Total length 6.8mm; head length 1.48mm; head width 1.2mm; cephalic index 81; scape length 1.02mm; scape index 85; eye diameter 0.80mm; pronotal width 0.86mm; and alitrunk length 2.1mm. Antennae 12 segmented with three segmented club. Whole body bright brownish yellow with scattered yellow hairs. Metanotum with a pair of long spines. Pronotum broader than long. Post petiole longer than broad (As given in Sheela & Narendran, 1998).

Materials examined: 1 Female, INDIA, Tamil Nadu, 20.iii.2007, Coll. Thresiamma Varghese and Ponnanna. 1 Female, INDIA, Karnataka, Indian Institute of Science Campus, 10.v.2004, Coll. Thresiamma Varghese.

Distribution: This species was known only from its type locality in Kerala. New distribution records are Karnataka (Indian Institute of Science Campus, Benagluru) and Tamil Nadu (Madurai Kamaraj University campus).

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Record of *Ulomimus indicus* Bates, 1873 (Coleoptera, Tenebrionidae) from south India

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ABSTRACT: *Ulomimus indicus* Bates, 1873 earlier known form east India and Sri Lanka in the Indian subcontinent is now recorded from south Indian states, Andhra Pradesh and Kerala. In addition, a brief description of the species along with illustrations are also given.
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KEY WORDS: Andhra Pradesh, Kerala, darkling beetles, diagnosis, rediscovery

Ulomimus Bates, 1873 is a small genus of darkling beetles with only two described species. It belongs to the tribe Ulomini of the subfamily Tenebrioninae. Bates (1873) described the type species *Ulomimus indicus* Bates, 1873 (Coleoptera, Tenebrionidae) from east India, without mentioning any specific locality. Schawaller (2019) transferred a species of *Cneocnemis* Gebien, 1914 to *Ulomimus* which became the second species of the genus. Since description of *U. indicus*, there are no other reports of the species from India. Liu *et al.* (2013) redescribed and illustrated the species including male genitalia and reported it from China, Indonesia and Thailand. Along with the rediscovery of *U. indicus* from India, some new distribution records are also reported for the first time.

The specimens from various surveys studied for morphological characters using LEICA® INVESTA 3 microscope. Male genitalia structures were dissected and kept in KOH (10%) for clearing and examined under LEICA® M 205A stereo zoom microscope and imaged using DFC 450 camera attached. Final illustrations were post-processed for contrast and brightness using Adobe® Photoshop CS6 software. Bouchard *et al.* (2021) was followed for classification. The specimens studied are deposited in the National Zoological Collections (NZC) of the Zoological Survey of India, Western Ghat Regional Centre, Kozhikode (ZSIK).

Systematic account

Subfamily Tenebrioninae Latreille, 1802;

Tribe Ulomini Blanchard, 1845;

Genus *Ulomimus* Bates, 1873

Ulomimus indicus Bates, 1873 (Figs. 1–8)

Ulomimus indicus Bates, 1873. *The Entomologist's monthly magazine*, 9: 202.

U. indicus Gebien, 1911. *Coleopterorum Catalogus*, 18: 399.

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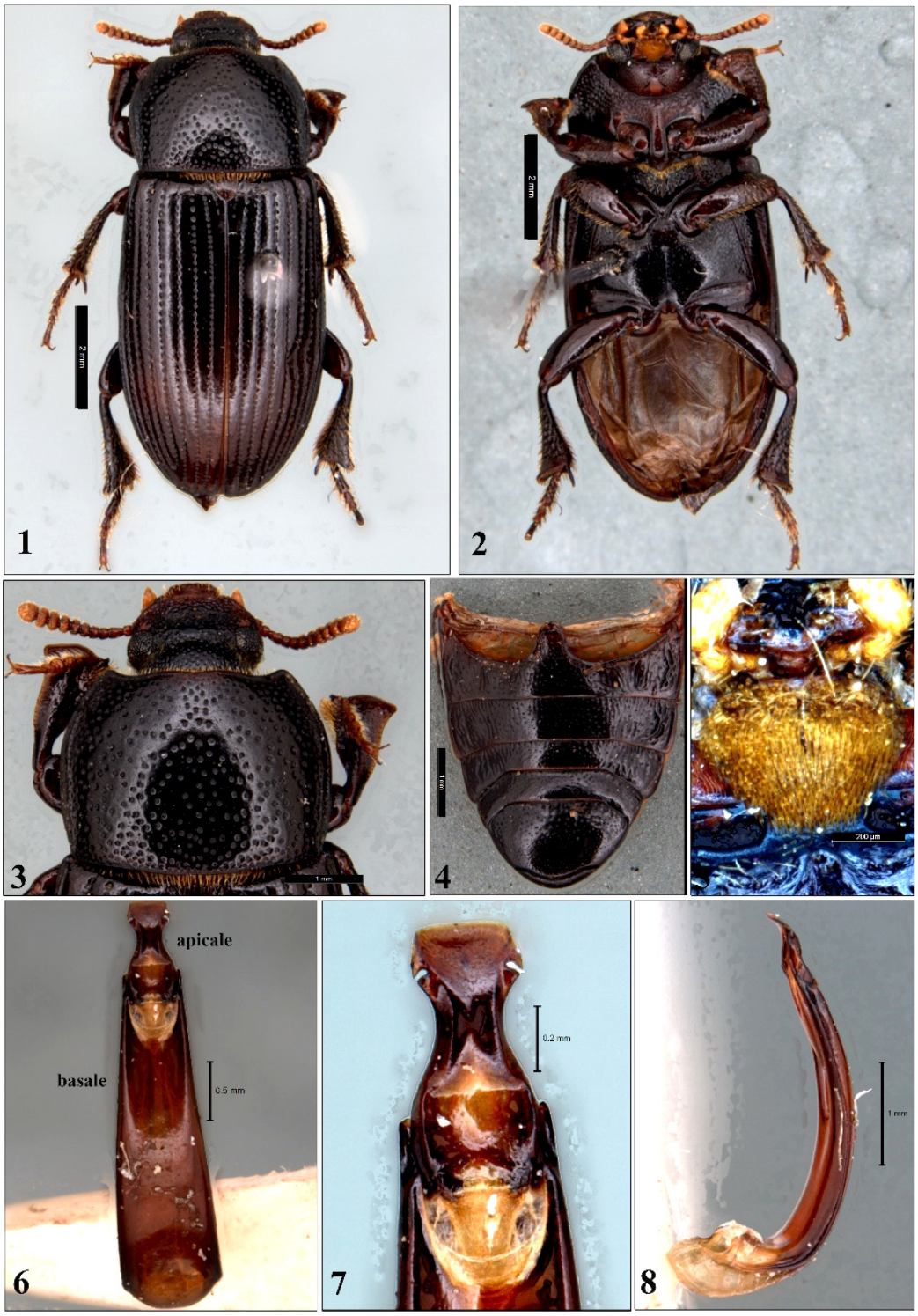

Figs. 1–8 *Ulomimus indicus:* 1. Habitus dorsal view, 2. Habitus ventral view, 3. Head and Pronotum, 4. Abdominal ventrites, 5. Mentum, 6. Aedeagus dorsal view, 7. Apicale showing oblique notch, 8. Aedeagus lateral view

Diagnosis. Male**.** Body length 8 mm. Body dorsally shining, black colored, subparallel sided, oblong, relatively convex (Fig. 1). Ventral side black shining, legs dark brownish, tarsi and antennae ferruginous (Fig. 2). Head coarsely punctured, transverse, clypeus coarsely rugopunctate, margin slightly emarginated in the middle. Labrum transverse, rectangular, rugose apically, sparse setae on the margins. Frons slightly raised with large punctures. Mentum heart shaped with dense yellow pubescence (Fig. 5). Antennae short with six-segmented club. Pronotum transverse, broad, 1.3 times as wide as long, coarsely punctured, punctures widely placed in middle, laterally becomes denser. Anterior margin emarginated, with dense hair fringes, anterior corner slightly raised, lateral margin rounded medially (Fig. 3). Elytra sub-parallel, striate-punctate, intervals slightly raised (Fig. 1). Protibiae expanded, outer edge sub apically with blunt angle, inner edge with dense hair fringes. Abdominal ventrites finely and sparsely punctated medially, laterally wrinkled, last ventrite apically grooved (Fig. 4). Aedeagus basale parallel sided but slightly diverging posteriorly, apicale broad constricted at the middle, oblique notch at posterior corner near the constriction (Figs. 6–8).

Materials examined: 1 Male, INDIA, Kerala, Wayanad, Punchavayal APC, 15.ii.2023, Coll. S. Sreejith & Party, ZSIK Regd. No. ZSI/WGRC/I.R.-INV.28493; 2 Females, INDIA, Kerala, Palakkad, Pattambi, 22.vi.2022, Coll. K.A. Sobhana, ZSIK Regd. Nos. ZSI/WGRC/I.R.-INV.28494–28495; 2 Males, 1 Female, INDIA, Andhra Pradesh, Srikakulam, 26.xi.2021, Coll. D. Gupta & Party, ZSIK Regd. Nos. ZSI/WGRC/I.R.-INV.28496–28498.

Distribution: India; East India (Bates, 1873); Kerala (New record), Andhra Pradesh (New record). *Elsewhere:* China, Indonesia, Sri Lanka, Thailand, Vietnam (Fairmaire, 1893; Kaszab, 1979; Liu *et al.,* 2013; Merkl, 1992).

Tenebrionidae is one of the poorly studied coleopteran families in India. One reason is their cryptic habits of the members of the family and lack of experts that currently work on this group. Sometimes earlier literature lacks specific information on distribution of the species, as in case of *U. indicus*, in the original description the distribution range is East India only. Extensive studies are required to understand the species richness and distribution of darkling beetles in India.

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Leptocorisa Latreille (Alydidae) species abundance and distribution in paddy fields of Thrissur, Kerala, India

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ABSTRACT: To understand the severity of *Leptocorisa* species (Hemiptera, Heteroptera, Alydidae) in the paddy fields, the adults and nymphal stages were collected during the agriculture season (November 2019 to March 2020). Sampling was done twice in the season, first in the panicle emerging stage and the second before harvest. The initial and final average densities of total insects varied from 1-4 individual m^{-2}. Two species of *Leptocorisa* were identified of which *L. oratoria* (F) was found to be the major pest in the paddy fields of Thrissur, where as *L. lepida* Breddin was relatively less prevalent.
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KEY WORDS: Rice bug, sucking, adult, nymph, *Leptocorisa oratoria, L. lepida*

Rice bugs are one of the few pests that feed directly on developing rice spikelet in the field, thus are the most recognized insect pests by farmers (Litsinger *et al*., 2015). They suck the sap from rice panicles in their milky stage, leaving the grains chaffy and smelly and, thus, reducing the crop production and quantity of rice. By sucking the milk, it reduces the grain content up to 40-60 per cent, leading to grain damage during milling, reduce the quality of rice (Argente and Heinrichs, 1983; Litsinger *et al*., 2015). The Reva district in Madhya Pradesh suffered 32 per cent of bug incidence during 2007 and 2008 (Tiwari *et al.*, 2014). Girish *et al*. (2015) reported 3-4 bugs/ hill from Dharwad, Karnataka, 0-3 insects/ hill from Allahabad (Bais *et al.,* 2017) and 15 insects/ 5 sweep nets from Varanasi (Padhan and Raghuram, 2018) were reported from Uttar Pradesh. It is important to know the distribution and abundance of major *Leptocorisa* species infesting paddy and their density to know their level of infestation for formulating management strategies. This study is to identify the species of *Leptocorisa* found in selected paddy fields of Thrissur District, Kerala and also to find out their density during the panicle emerging stage and just before rice harvest.

Paddy fields were selected randomly. Insects were collected from selected paddy fields of Velur (10.645^0N; 76.170^0E), Mundathikkode (10.646^0 N; 76.193^0E), Thangalur (10.612^0N; 76.184^0 E), Padiyoor (10.303^0N; 76.184^0E) and Mullassery (10.530^0N; 76.078^0E) of Thrissur district, Kerala, during the agricultural season November 2019 to March 2020. Insects were collected from two plots in each field. Total area collected varied in each field- Velur (17.64 m^2), Mundathikkode (20.88 m^2), Thangalur (15.84 m^2), Padiyoor (16.02 m^2) and

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Mullassery (13.86 m^2). The insects were collected along five transects. Four transects were along the field edges and one inside the field. Along each transect, insects were collected from a regularly spaced sampling point. An entomological sweep net of 0.18 m^2 was used for collecting insects. The number of sampling points varied according to the area of the plot. Insect collection was done two times, once during the panicle emergence and once during grain maturing stage. Only *Leptocorisa* species were collected from the sweep net by visual observation. The collected insects were identified. Identification was done using standard taxonomic keys (Ahmad, 1965; Dolling, 2006; Lahari *et al.,* 2024). Images of insects collected in different parts were taken using the Leica S8AP0 microscope with 8:1 zoom and 300 Lp/mm. Total insect density, individual insect density, adults and nymphal densities were calculated separately using the plot's total area from the collected insects. One-way ANOVA was performed for the total insect densities.

A total of 377 insects were collected during the rice panicle emergence stage and before harvest. Two species of *Leptocorisa* (Hemiptera, Heteroptera, Alydidae) were identified. One is the major pest of rice, *L. oratoria* (Fabricius,1764) with brownish-black ventro-lateral spots on the abdomen (Ahmad, 1965) and curved paramere, tapering to point at the apices in males (Lahari *et al.,* 2024) (Figs. 1-3).

Leptocorisa oratoria (84%), is a major pest of lowland wet rice areas (Siwi and Van Doesburg, 1984), of Thailand, Vietnam, Philippines, India (Hasegawa, 1971), China, Japan (Dolling, 2006) and in Iran (Ghahari *et al*., 2010). *L. lepida* (Breddin) (Figs. 4 - 6) with long cylindrical and pointed paraclypea (Ahmad, 1965) characterized by very short and slime body with dark endocorium of hemielytra, and blade-like paramere, curved with pointed apices in males (Lahari *et al.* 2024) is usually found in association with weed grass near paddy fields.

Both species of *Leptocorisa* were found in Velur, Mundathikkode and Thangaloor. In Padiyoor and Mullassery only *L. oratoria* was found, *L. lepida* was absent.

The average densities of total insects (average density = total number of insects collected during panicle emerging stage (Initial) and just before harvest (Final)/ total sampling points X area of net opening) collected from each plot during the panicle emerging stage (Initial) and just before harvest (Final) showed statistically significant differences ($p < 0.05$). The initial and final average densities of total insects varied from to one- four individuals/ m^2. A decrease in final density was found in all fields except in Padiyoor. In Padiyoor the total density increased from 1.996 to 2.556 individuals/ m^2 (Fig. 7). A study by Kandibane (2008) for phytophage Hemiptera pointed out that these kinds of insects prefer weeded plots, especially *Echinicloa colonum, E. crus-galli* act as a secondary host for *Leptocorisa* spp. Insect density in Padiyoor increased during the final stage may be due to the presence of their secondary host plant in the field. In all the fields, *L. oratoria* (both adult and nymph) density was higher than *L. lepida. Leptocorisa oratoria* density was found to decrease towards the final stage. These observations were in support with the previous studies of Ghule *et al.* (2008) in Gujarat on *Leptocorisa acuta* and Girish *et al*. (2012) in Mugad, Karnataka. *Leptocorisa oratoria* density found to be more than the economic threshold level in most sites as per the Integrated pest management package for rice (Prakash *et al.,* 2014). In other places *L. lepida* densities also found to be decreased in the final stage (Fig. 8). Adult and nymphal stages of *L. oratoria* indicate that adult density was similar during initial and final stage. Nymphs were abundant during the panicle emerging stage and following the grain maturation the nymphal densities decreased (Fig. 9). The results are supporting with the previous work done by Girish *et al*. (2015). *Leptocorisa lepida* (15%) was only found in Velur, Mundathikkode and Thangaloor. In these fields, initial densities of adult *L. lepida* was higher than the final. Only from Velur field *L. lepida* nymphal stages found. From other fields *L. lepida* nymphal stages were not found (Fig. 10).

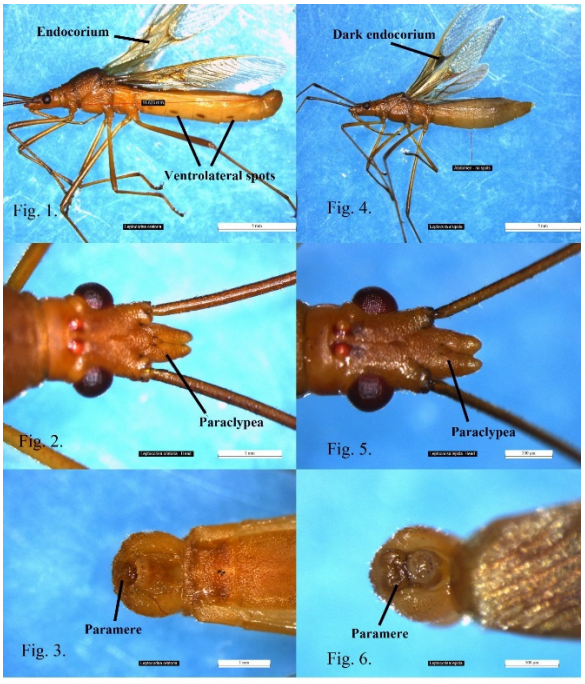

Fig.1 *Leptocorisa oratoria* (Fabricius, 1764) (Male), Fig.2 *L. oratoria* - Head, Fig.3 *L. oratoria*- Terminalia dorsal view, Fig. 4 *L. lepida* (Breddin) (Male), Fig. 5 *L. lepida*- Head, Fig. 6 *L. lepida* -Terminalia dorsal

Corbett (1930), mentioned about adult *L. lepida* as a species that prefer grasses. From this study, it is concluded that the major *Leptocorisa* species is found to be *L. oratoria*. Proper field practices and pest management activities needed to be taken to control these pests.

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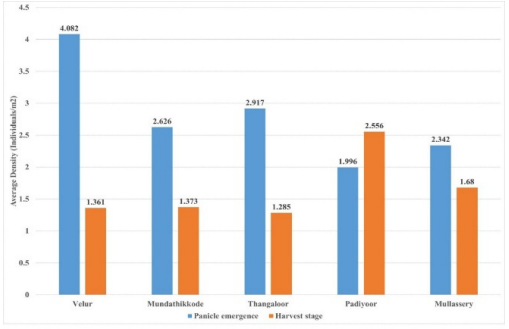

Fig. 7.

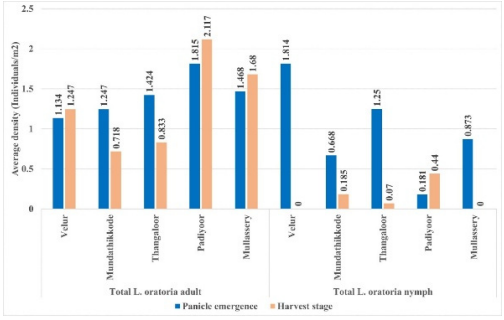

Fig. 9

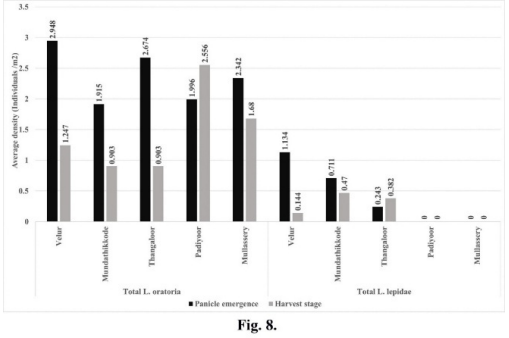

Fig. 8.

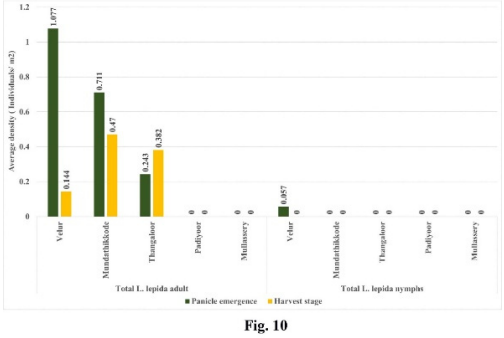

Fig. 10

Fig. 7 Average Density of Total Insects, Fig. 8 Average densities of total *L. oratoria* and *L. lepida*, Fig. 9 Average densities of *L. oratoria* adults and nymphs, Fig.10 Average densities of *L. lepida* adults and nymphs

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Report on the rediscovery of *Thacona ferreopicta* (Hampson, 1900) (Lepidoptera, Scranciidae) with the first description of female genitalia from India

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ABSTRACT: Scranciidae Miller, 1991 comprises of three genera from India including *Phycidopsis* Hampson, 1893, *Gargetta* Walker, 1865 and *Thacona* Walker, 1865. Out of the three species, *Thacona ingens* (Walker, 1866), *T. punctifascia* (Hampson, 1897) and *T. ferreopicta* (Hampson, 1900), only *T. ferreopicta* is known from south India. Though reported previously, very little is known about its distribution status. The present study puts forth the report of the moth, *T. ferreopicta* from India, from Alakkad, Kannur District, Kerala for the first time. The description of female genitalia with image is provided for the first time. The first live habitus photograph of the species along with details of the specimens of *T. ferreopicta* in the holdings of the Natural History Museum, London (NHMUK) has been incorporated. In addition to the type specimen from Sri Lanka, there are specimens from Meghalaya and south India in NHMUK. However, our present understanding of the distribution of *T. ferreopicta* is from these museum specimens alone. This study provides information based on the female specimen and its genitalia collected from Kerala. The male genitalia of the species remain unknown and requires further investigation. This study highlights the significance of extensive field surveys needed throughout the region for a better understanding of the diversity and distribution of moths. In addition, this paper provides a brief account of Scranciidae Miller, 1991 along with the checklist of the Scranciid moths from India.
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KEY WORDS: New record, Noctuoidea, Notodontidae, Alakkad, checklist

The genus *Thacona* was described by Walker in 1865, with *T. costivitta* Walker, 1865 as its type species and was then a monotypic genus. The genus was formerly included in the family Notodontidae. Scranciidae Miller, 1991, which was until recently considered a subfamily of Notodontidae, has been attributed family status by St Laurent *et al*. (2023) based on phylogenomic evidence. The adults of the family are characterized by narrow forewings with the margins either straight or convex. The hindwings are almost triangular. The coffee-bean-shaped signum in the corpus bursae of females is a characteristic feature of Scranciidae (St Laurent *et al*., 2023). Scranciidae shows a wide distribution from Africa to Australasia (Holloway, 1983; Kiriakoff, 1964; Schintlmeister,

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2008, 2020; Schintlmeister and Witt, 2015). In India, eight species in three different genera are recognised in the Scranciidae; four species in genus *Gargetta* Walker, 1865, three species in *Thacona* and a single species in genus *Phycidopsis* Hampson, 1893.

Adults of *Thacona* are medium-sized (wingspan ~ 40-50mm). Males have bipectinated antennae whereas females have filiform antennae. The forewings are elongated and narrow in both cases. A well-defined discal spot with a pale border, a black costal patch, and a row of black dots identifying the marginal fascia usually make up the forewing maculation. Furthermore, fuscous dorsal spots near the base and fuscous tornal spots distally are also present. The submarginal region of the pale brown hindwing is fuscous. With the exception of *Thacona benderi* (Schintlmeister, 1981), *Thacona* male genitalia have a rather tiny and slender uncus (Schintlmeister, 2022). Socii are absent. Valvae are elongated and the costa is heavily sclerotized. Both valva arms have asymmetric harpes, which are either bifurcate or trifurcate. The phallus is fairly short and straight. The anterior margin of the eighth abdominal tergum is bilobed. In the female genitalia apophyses are short, antrum is relatively large and the ductus bursae is elongated, corpus bursae is pyriform and has a highly sclerotized reniform signum (Schintlmeister, 2022).

The present paper describes the female genitalia of *T. ferreopicta* Hampson, 1900 for the first time, as well as documentation of recent collecting localities for this otherwise rarely reported species. This is a new record of the above said species from Kerala. However, information on the male genitalia of this species remains unknown and should be targeted in future sampling efforts, as should photographic documentation of the poorly known life history of this species. The present study also provides the first complete checklist of Scranciidae from India due to previous omissions. Due to the lack of robust locality information for many of these species in India, extensive field surveys are necessary to provide a better understanding regarding the diversity and distribution of the group in the region.

Individuals for the present study were observed on two occasions near an agricultural environment from Alakkad (12°14’44"N; 75° 24' 10"E), a lush green village neighbouring Payyanur municipality in Kannur district, of North Kerala. The first sighting was on the 20th of August 2021 and the individual was photographed but not collected (Fig. 1). A second individual was observed on the 5th of the same month, a year later. This specimen was collected and was kept for further studies (Fig. 2).

The identification of the specimen was determined based on the original description (Hampson, 1900). *Thacona ferreopicta* was distinguished from *T. ingens* in being darker without the pale brown suffusion in the forewing and *T. punctifascia* in the presence of purplish blotches and being paler. The identified specimen was duly registered and deposited in the insect collection Lab of The Department of Zoology, Payyanur College, Kannur, Kerala. The genitalia dissection procedure of the same individual followed Robinson (1976). The dissected genitalia are preserved in vials containing a mixture of alcohol and glycerol in 3:1 ratio. The photograph was taken under a compound microscope (Fig. 4).

Systematic accounts: Family Scranciidae Miller, 1991; Genus *Thacona* Walker, 1865

Thacona ferreopicta (Hampson, 1900): 40 (*Gargetta ferreopicta*).

Type Locality: Ceylon [Sri Lanka]

Distribution: Sri Lanka, India; Meghalaya, Karnataka, Kerala.

Material examined**:** 1♀**,** India, Kerala, Kannur District, Alakkad (12°14’44"N; 75°24’10"E), 05. VIII. 2022. Coll. M.P. Unnikrishnan.

Female description**:** Antenna filiform. Head and thorax reddish brown, abdomen pale brown; covered in darker scales terminally. Forewing length: 23mm. Forewing pale brown, suffused with reddish brown scales. Forewing pale brown, suffused with reddish brown scales. Costa and inner margin characterized by dark patches in antemedial and postmedial region. A dark spot present in the

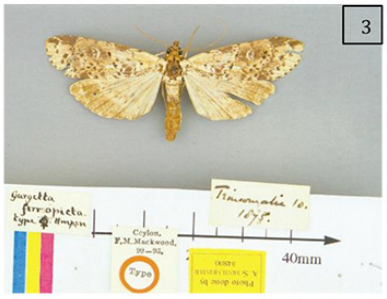

Fig.1 *Thacona ferreopicta* live habitus photograph. Fig. 2 *T. ferreopicta* female specimen in the present study. Fig. 3 *T. ferreopicta,* holotype ♀ from NHMUK. Fig. 4 *T. ferreopicta* Female genitalia (Scale bar = 1mm).

sub-basal region. Antemedial line present, angled below costa. Medial area characterized by two vertical specks along median nervure followed by a curved medial line. The discocellular region is suffused with reddish brown and a characteristic reniform spot. Traces of a dark postmedial line present on either side reniform spot. Black specks present along a diffuse submarginal band submarginally. Wing margin with further brown specks between vein termini. Hindwing pale brown with dark brown postmedial band extending towards margin. Female genitalia (Fig. 3) with setae covered papillae anales, apophyses posteriores shorter than VIII segment length and longer than apophyses anteriores; proximal half of ductus sclerotized, cylindrical, corpus bursae contain a signum.

Thacona ferreopicta was described by Hampson in 1900 from Sri Lanka. There are specimens of *T. ferreopicta* in the holdings of NHMUK from Khasi Hills (Meghalaya, India) and north Kanara (south India). However, this species was not listed in the subfamily Scranciinae in the catalogue of Indian Notodontidae (Chandra *et al*., 2018).

Checklist of Scranciidae Miller, 1991 from India

1. *Phycidopsis albovittata* Hampson, 1893: 91.

Type locality: Ceylon [Sri Lanka]

Distribution: Cambodia, China (Taiwan), Indonesia (Java, Sulawesi), Japan, Philippines, Sri Lanka, Thailand¸ India; Assam, Arunachal Pradesh, Meghalaya, Karnataka (Chandra *et al*., 2018).

2. *Gargetta costigera* Walker, 1865: 455.

Type locality: Darjeeling [West Bengal, India]

Distribution: Bhutan, China, Nepal, Thailand, India; Himachal Pradesh, Uttarakhand, West Bengal, Sikkim, Assam, Meghalaya, Chhattisgarh, Andaman Islands (Chandra *et al*., 2018).

3. *Gargetta curvaria* Hampson, 1892: 136.

Type locality: Darjeeling [West Bengal, India]

Distribution: China (Taiwan), Indonesia (Borneo, Sulawesi, Sumatra), Myanmar, Philippines, Thailand, Vietnam, India; West Bengal, Sikkim, NE India (Chandra *et al*., 2018).

4. *Gargetta divisa* Gaede, 1930: 615.

Type locality: Malang [Java, Indonesia]

Distribution: China, Indonesia (Java, Sumatra),

Laos, Malaysia, Myanmar, Philippines, Thailand, Vietnam, India; Sikkim, Assam, Arunachal Pradesh, Andaman Islands (Chandra e*t al.,* 2018).

5. *Gargetta nagaensis* Hampson, 1892: 135.

Type locality: Naga Hills [Nagaland, India]

Distribution: China, Myanmar, Thailand, India; Assam, Nagaland (Chandra *et al*., 2018).

6. *Thacona ingens* (Walker, 1866): 1823 (*Porsica ingens*)

Type locality: Sylhet [Bangladesh]

Distribution: Bangladesh, China, Indonesia, Myanmar, Thailand, Vietnam, India: Sikkim, Assam (Chandra *et al*., 2018).

7. *Thacona punctifascia* (Hampson, 1897): 281 (*Gargetta punctifascia*)

Type locality: Khasis [Meghalaya, India]

Distribution: China, Indonesia (Borneo, Java, Sumatra), Laos, Malaysia, Philippines, Thailand, Vietnam, India: Assam, Meghalaya (Chandra *et al*., 2018).

8. *Thacona ferreopicta* (Hampson, 1900): 40 (*Gargetta ferreopicta*)

Type locality: Ceylon [Sri Lanka]

Distribution: Sri Lanka, India; Meghalaya, Karnataka and Kerala.

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New records of egg parasitoids (Hymenoptera, Chalcidoidea, Trichogrammatidae) from Kashmir Valley, Jammu and Kashmir, India

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ABSTRACT: Extensive surveys carried out in a range of habitats in several Kashmir valley districts, documented ten species of trichogrammatids from genera, including *Chaetostricha* Haliday, *Lathromeroidea* Girault, *Oligosita* Haliday, *Paracentrobia* Howard, *Pseudoligosita* Girault, *Trichogramma* Westwood and *Ufens* Girault, as indigenous species with newly identified distribution in the Kashmir valley. These findings expand the known diversity of these egg parasitoids and mark the first report of these trichogrammatids that have been recorded in Kashmir valley, Jammu and Kashmir, India.
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KEY WORDS: Surveys, indigenous species, distribution, Western Himalayas

The Trichogrammatidae family is a significant taxon within the Chalcidoidea superfamily. These insects, which may be solitary or gregarious, are egg parasitoids. They aim at an extensive range of insects from multiple orders, specifically emphasizing Coleoptera, Diptera, Hemiptera and Lepidoptera. They aim at an extensive range of insects from multiple orders, specifically emphasizing Coleoptera, Diptera, Hemiptera, and Lepidoptera(Pinto and Stouthamer, 1994). In India, however, there are about 200 species across 35 genera, distributed among two subfamilies and five tribes (Noyes, 2022). The research was conducted in the Kashmir valley, located in Jammu and Kashmir, India. This valley is encircled by the Himalayan mountains, bordered by the Pir Panjal Range in the southwest and the Greater Himalayan Range in the northeast.

The collection sites of Kashmir Valley, J&K, India are located in the districts of Ganderbal, Srinagar, and Bandipora. Used a sweeping net for collecting insect samples from different habitats, including grass fields, rice paddies, vegetable gardens, orchards, herbs, shrubs, and bushes, across various locations in the Ganderbal, Srinagar, and Bandipora districts of the Kashmir Valley. The samples were immediately transferred into small vials containing ethyl alcohol (70%) for preservation. Freshly collected trichogrammatid specimens were sorted under a stereo zoom microscope (Olympus SZX16) and thereafter preserved in Eppendorf tubes with

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70 per cent ethyl alcohol. Slides were prepared according to Noyes' (1982) methodology. Dissections were done using the stereo zoom microscope (Olympus SZX16), while photography and measurements were carried out with a CILIKA Patented Digital Head Technology slide microscope, equipped with an 8-megapixel camera on an iPad. All specimens were identified up-to species level with help by studying the world as well as Indian literature, and keys provided by Nagaraja and Nagarkatti (1973) and Yousuf and Shafee (1988).

This study represents the first systematic examination of trichogrammatids in the Kashmir valley. It includes new records of species from the Trichogrammatidae family, specifically within the genera *Oligosita* Haliday, *Pseudoligosita* Girault, *Paracentrobia* Howard, *Lathromeroidea* Girault, *Ufens* Girault, *Chaetostricha* Haliday and *Trichogramma* Westwood. *Chaetostricha magniclavata* Yousuf and Shafe, *Lathromeroidea ajmerensis* Yousuf, *L. longiciliata,* Ikram &Yousuf, Shafee *Oligosita gilvus* Yousuf & Shafee*, O. ferozepurensis* Ikram & Yousuf, *O. meerutensis* Yousuf & Shafee*, Paracentrobia bharatpurensis* Yousuf and Shafee, *Pseudoligosita nephotetticum* (Mani)*, Trichogramma chilonis* Ishii and *Ufens latipennis* Yousuf & Shafee are new records.

1.*Pseudoligosita nephotetticum* (Mani, 1939)

P. nephotetticum (Mani, 1939)

P. nephotetticum (Mani): Pinto & Viggiani (2004)

Host: *Nephotettix bipunctatus* (Uhler) on rice (Hemiptera: Cicadellidae).

Distribution: INDIA: Bihar, Chhattisgarh (Raipur & Bilaspur: Ikram *et al.*, 2023), Haryana (Bhiwani, Hisar, Jhajjhar, Karnal and Kurukshetra: Yousuf and Ikram, 2015), Jharkhand, Madhya Pradesh, Punjab, Rajasthan, and Uttar Pradesh (Noyes, 2022).

Material examined. INDIA: Jammu and Kashmir: Ganderbal, Wakura; 4♀, 1♂, 1.vi.2023; Zazna, 2♀, 4.vi.2023 coll. Rabiya Irshad (sweeping). Srinagar, Harwan; 2♀, 15.vii.2023; Hazratbal; 2♀ and 1♂, 17.vii.2023; coll. Rabiya Irshad (sweeping). Bandipora: Sumbal; 2♀, 1. viii.2023; Tarazuea; 2♀, 6.viii.2023; coll. Rabiya Irshad (sweeping).

2. *Oligosita meerutensis* Yousuf and Shafee; Yousuf and Shafee (1984)

Host: Unknown

Distribution: INDIA: Haryana, Jharkand, Punjab, Uttarakhand (Dehradun, Almora: Begum *et al.,* 2015) and Uttar Pradesh (Meerut).

Material examined. INDIA: Jammu & Kashmir: Ganderbal: Gadoora; 1♀, 3.vi.2023; Nuner; 1♀, 6.vii.2023; coll. Rabiya Irshad (sweeping). Srinagar: Eidgah;1♀, 16.vii.2023; coll. Rabiya Irshad (sweeping).

3. *O. gilvus* Yousuf and Shafee; Yousuf and Shafee (1984)

Host: Unknown

Distribution: INDIA: Assam (Kamrup: Begum *et al.*, 2015), Punjab, Madhya Pradesh, Uttar Pradesh.

Material examined. INDIA: Jammu and Kashmir: Ganderbal: Gadoora; 1♀, 3.vi.2023; coll. Rabiya Irshad (sweeping); Nuner; 1♀, 6.vi.2023; coll. Rabiya Irshad (sweeping); Srinagar: Gulabpora ; 1♀, 18.vii.2023; coll. Rabiya Irshad (sweeping).

4. *O. ferozepurensis* Ikram and Yousuf (2016)

Host: Unknown

Distribution: INDIA: Delhi, Haryana, Punjab, Uttar Pradesh.

Material examined. INDIA: Jammu & Kashmir: Ganderbal: Rapora; 1♀, 5.vi.2023; Nuner; 1♀, 6.vi.2023; coll. Rabiya Irshad (sweeping). Srinagar: Shalimar; 1♀, 20.vii.2023; coll. Rabiya Irshad (sweeping). Bandipora: Shadipora; 1♀, 8. viii.2023; Hajan; 1♀, 9.viii.2023; coll. Rabiya Irshad (sweeping).

5. *Lathromeroidea ajmerensis* Yousuf and Shafee (1988)

Host: Unknown

Distribution: INDIA: Chhattisgarh (Bilaspur: Ikram *et al.*, 2023), Madhya Pradesh,

Punjab, Rajasthan.

Material examined. INDIA: Jammu & Kashmir: Ganderbal: Rapora; 1♂, 5.vi.2023;

Nuner ;1♂, 6.vi.2023; coll. Rabiya Irshad (sweeping).

6. *Paracentrobia bharatpurensis* Yousuf and Shafee; Yousuf and Shafee (1988)

Host: Unknown

Distribution: INDIA: Rajasthan (Bharatpur), Punjab.

Material examined. INDIA: Jammu and Kashmir: Ganderbal: Manasbal; 1♀, 7. vi.2023;

1♀, Wakura; 1.vi.2023; coll. Rabiya Irshad (sweeping).

7. *Chaetostricha magniclavata* Yousuf and Shafee; Yousuf and Shafee (1988)

Host: Unknown

Distribution: INDIA: Uttar Pradesh.

Material examined. INDIA: Jammu and Kashmir: Nowgam; 1♂, 19.vii.2023; coll. Rabiya

Irshad (sweeping).

8. *Lathromeroidea longiciliata,* Ikram and Yousuf; Ikram and Yousuf (2017)

Host: Unknown.

Distribution: INDIA: Punjab (Jalandhar, Nawanshahr), Uttar Pradesh.

Material examined. INDIA: Jammu and Kashmir: Ganderbal: Rapora; 1♀, 5.vi.2023; Nuner; 1♀, 6.vi.2023; coll. Rabiya Irshad (sweeping). Bandipora: Shadipora;1♀, 8. viii.2023; coll. Rabiya Irshad (sweeping).

9. *Trichogramma chilonis* Ishii (1941)

Host: *Achaea janata, Acherontia styx, Acigona steniellus, Acrobasis caryae, A. juglandis, Aglossa dimidiata, Agraulis vanillae, Agrius cingulata, Agriusless convolvuli, Ampillia dioscoridea, Anomis flava, Arctiaas coerulea, Argyroploce schistaceana, Ascotis selenariadianerla, Atherigona soccata, Bactra sp., Barathra Agrius convolvuli brassicae, Cerura vinula, Chilo indicus, C. infuscatellus, C. partellus, C. sacchariphagus, C. suppressalis, C. venosatus, Clanis bilineata, Clostera anachoreta, Cnaphalocrocis medinalis, Cocytodes coerulea, Corcyra cephalonica, Creatonotus transiens, Crocidolomia binotalis, Danaus plexippus, Deilephila nerii, Diatraea saccharalis, Earias insulana, Earias vitella, Emmalocera depressella, Trichogramma breviciliata, Ephestia cautella, Ergolis merione, Etiella zinekenella, Eucosma schistaceana, Euproctis flavinata, Eutectona machaeralis, Gastropacha populifolia, G. glycinivorella, Helicoverpa armigera, Heliothis assulta, H. zea, Hemerophila atrilieneata, Herse convolvuli, Homona coffearia, Hyblaea puera, Hymenia recurvalis, Jaspida distinguenda, Laspeyresia caryana, Mycalesis gotama, Naranga aenescens, Oebia undalis, Olethreutes schistaceana, Ostrinia furnalis, O. nubilalis, Papilio xuthus, Parasa consocia, Parnaraentire guttata, Pelopidas mathias, Philosamia cynthia ricini, Pieris rapae, Plutella xylostella, Procera sacchariphagus, Procera, Corcyra cephalonica, Eutectona machaeralis, venosatus, Prodenia litura, Prodesaia kurosawai, Psara, Hyblaea puera, Hasora alexis, Samia cynthia, Scirpophaga excerptalis, S. incertulas, S. innotata, S. nivella, Scirpophaga sp., Sesamia inferens, Sitotroga cerealella, Spilarctis obliqua, Spodoptera litura, Spodoptera* (Khan, 2002).

Distribution: INDIA: Andhra Pradesh, Bihar, Delhi, Gujarat, Haryana (Kumar *et al.,* 2009; Ram *et al.,* 1998; Ahmad *et al.,* 1997), Himachal Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Punjab, Rajasthan, Tamil Nadu, Uttarakhand, Uttar Pradesh, and West Bengal.

Material examined. INDIA: Jammu and Kashmir: Ganderbal: Gadoora; 1♂, 3.vi.2023; Nuner; 1♂, 6.vi.2023; Nowgam; 1♂, 19.vii.2023; coll. Rabiya Irshad (sweeping). Bandipora: Asham;1♀(on slide), 3. viii.2023; coll. Rabiya Irshad (sweeping).

10. *Ufens latipennis* Yousuf and Shafee; Yousuf and Shafee (1988)

Host: Unknown

Distribution: INDIA: Uttar Pradesh

Material examined. INDIA: Jammu and Kashmir: Ganderbal: Wakura; 1♀, 1.vi.2023; coll. Rabiya Irshad (sweeping).

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OBITUARY

Dr. K.S.S. NAIR

1938-2025

K.S. SADASIVAN NAIR *alias* Krishna Sivasankara Sadasivan Nair, former Forest Entomologist and Director, Kerala Forest Research Institute, Peechi, passed away on the 20th April, 2025, at his residence 'Saketh' in Sasthamangalam, Thiruvanathapuram. He was an astute scientist and administrator who made substantial contributions to tropical forest insect pest management. He was 87.

Dr. K.S.S. Nair was born on 18-08-1938 as the youngest child of Shri. K. Sivasankara Pillai and Smt. Madhavi Amma of Kalluvila family in Thiruvanathapuram. Late Dr. S.M. Nair, founder Director of National Museum of Natural History, New Delhi was his elder brother. KSS had his early education in the Govt. Model Higher Secondary School for Boys, Chalai, Thiruvanathapuram from where he passed his matriculation in 1954 in First Class with distinction. Thereafter, he joined the Intermediate College, Thiruvananthapuram (now University College), and completed his Pre University (1956) and B. Sc. (1958) degree courses in First Class with Distinction. As a student, KSS was very active in sports and extracurricular activities. He has represented the college in Inter-University Volleyball tournaments and

won several prizes. KSS also showed keen interest in learning various languages. He passed a certificate course in Hindi (Rashtra Bhasha Pariksha) conducted by Dakshin Bharat Hindi Prachar Sabha in 1951 and also completed a Certificate Course in German Language in1962. With regard to English, he had excellent mastery of the language and he was an excellent orator, writer and editor.

Dr. Nair completed his M.Sc. (1960) and Ph.D. (1964) from the Department of Zoology, Maharaja Sayajirao University of Baroda, India. For his Ph.D., he worked on a topic entitled 'Studies on certain aspects of the physiology of the furniture-carpet beetle, *Anthrenus vorax* Waterhouse (Dermestidae, Coleoptera)' under the guidance of the eminent Zoologist Dr. J.C. George. On completion of Ph.D., he joined as a Lecturer in Zoology (1964-1969) in the same University. During this period, he also served as the Principal Investigator of a PL480 project to elucidate the cause-effect relationship of diapause in the stored grain pest, khapra beetle, *Trogoderma granarium* Everts.

On securing a post-doctoral Fellowship in the Department of Environmental Biology, University of Guelph, Ontario, Canada, KSS left India in 1970, to work on the biology, ecology, and chemical basis of host plant selection of the seed corn maggot complex, *Hylemya* (*Delia*) *platura* and *H*. (*Delia) liturata* (Diptera: Anthomyiidae) under the supervision of Prof. F. L. McEwen (1970 to 1975). As part of his post-doctoral programme, he also got trained in the Systematic Identification of Diptera with special reference to Anthomyiidae under the guidance of Dr. J.P. McAlpine of the Agriculture Department, Ottawa, Canada. While in Canada, he was President of Indian Students Association, University of Guelph from June 1971 – April 1972· In this capacity, he held monthly meetings of the Indian student community in the University and their families to discuss common issues and also organized various social and cultural activities.

On completion of his assignment in Canada, Dr. Nair returned to India in December 1975 and joined the Kerala Forest Research Institute as a Scientific Pool Officer, supported by the Council of Scientific and Industrial Research (CSIR), Government of India. At that time, KFRI was in its formative stage, and Dr. Nair was selected as its first Entomologist. On 11th Dec. 1976, he took charge as the Head of the Division of Entomology when the Division was just established. With five other Entomologists recruited subsequently, *viz.,* Dr. R.V. Varma, Dr. George Mathew, Dr. V.V. Sudheendrakumar, Dr. K. Mohanadas and Dr. T.V. Sajeev, he carried out problem-solving research as well as pioneering fundamental studies on forest insect fauna, pest ecology and population dynamics to provide practical methods of pest management for use by the Forest Department. The results of these investigations were published in over 300 publications including Journal Papers, Chapters in Books, Reviews, Information Bulletins, Research Reports, and Books. These studies also received international recognition as it extended our knowledge on various aspects of tropical Forest Entomology. Based on his contributions to tropical forest pest management, Dr. Nair was elected as Co-Chairman and then as Chairman of the IUFRO (International Forestry Research Organizations) Working Party S2.07.07 entitled 'Protection of forest in the tropics' (later renamed 'Forest Health'), for the period 1986 to 1996. He continued to head the Division of Entomology for 18 years. During this period, he also held additional charge of the Director of the Institute for three years from 1988 to 1991. Later in Jan.1995, he was appointed as the Director of KFRI and continued until superannuation in July 1999.

Dr. Nair was quite philosophical and at the same time very practical in his research approach. He followed an ecosystem-based approach in tackling forest pests. He carried out several studies to elucidate the population dynamics of insect pests in natural forests and in plantations. Do plantations suffer greater

pest damage than natural forests? And if so, why? This aspect was investigated in relation to teak defoliator (*Hyblaea puera* Cramer) epidemics in teak plantations in Kerala. The occurrence of migratory and non-migratory phases has been considered as the cause for seasonal epidemics in teak defoliator. He also recognized the role of nuclear polyhedrosis virus (NPV) in natural suppression of teak defoliator. Based on these findings, application of *Hp*NPV control agents within an integrated pest management strategy has been proposed against the teak defoliator.

Another major contribution of Dr. Nair was pertaining to termite control in Eucalypt plantations. In young plantations of eucalypts, termites cause serious damage due to root feeding. Though a number of control methods have been proposed, there had been no attempt to systematically evaluate them under Indian conditions. Combinations of selected insecticides in different dosages, formulations and methods of application were tested in several experiments and spot application of appropriate chemicals have been proposed.

Biology and control of insect pests of fast-growing hardwood species particularly *Paraserianthes (Albizia) falcataria* and *Gmelina arborea*; seasonal Incidence, host Range and control of the Teak Sapling Borer, *Sahyadrassus malabaricus* as well as protection of fibrous raw material (reed and bamboo) in storage against deterioration by biological organisms are some other major research projects handled by him.

After his retirement from the service of KFRI, Dr. Nair worked as a Visiting Scientist at CIFOR (Center for International Forestry Research), Bogor, Indonesia from Sept. 1999 to Dec. 1999. In this capacity, he made an assessment of the insect pests and diseases situation in Indonesian forests and examined the susceptibility of plantations of exotic versus native tree species to pest outbreaks. From October 2002 to December 2004, he worked as an Emeritus Scientist Under the Department of Science and Technology, Government of India. Under this programme, he prepared the manuscript of a book entitled 'Tropical Forest Insect Pests: Ecology, Impact, and Management' which was published by Cambridge University Press in May 2007. A paperback edition of this was published later in October 2012. The book gives comprehensive information on all aspects of tropical forest insects - their diversity, ecology, circumstances in which their populations increase to assume pest status, pest population dynamics in plantations and natural forests and plantations of exotic *versus* native species, and gives detailed information on specific insect pest problems of common plantation tree species across the tropics and recommendations for their management. During this period he has also served as a Member of the Research Council of KFRI besides being a member in several State and National Expert Committees.

Dr. Nair who was a Life Member of the Association for Advancement of Entomology (AAE) since 1975 took charge as President of the Association in October 1998 and continued in this position till March 2019. During this period, he also served as the Editor of the journal, ENTOMON. It is noteworthy that during his tenure, he worked hard to bring out all pending issues of the journal.

Dr. Nair was a committed scientist and at the same time a good administrator. He was very soft and kind and at the same time, highly ethical and idealistic in nature and never yielded to any unjust pressure or recommendation. He was a unique personality who took great pleasure in helping his colleagues and students in the scientific pursuits and readily helped anyone in distress. Dr. Nair's contributions to forest entomology and his vision in shaping the destiny of KFRI to an Institution of Excellence in Tropical Forestry will always be appreciated.

Dr. Nair was a quiet, and an unassuming person but his intellectual superiority was obvious to those who worked with him. The depth of his knowledge was astounding. Above all, he was a keen and intelligent observer of nature with a deep knowledge of many fields.

Dr. Nair was a very kind, affectionate and family-oriented person. His demise will be a great loss to his wife, Smt. Sathi Nair, children and grandchildren. In fact, his wife's interest in his work and the strong support she gave throughout their happy married life contributed substantially to Dr. Nair's success in scientific pursuits. I am sure, Dr. Nair's life and work will remain to inspire others and he will be remembered with affection by all those who knew him.

Dr. George Mathew,
Former Principal Scientist & Head,
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Kerala Forest Research Institute,
Peechi-680653, Kerala.

AUTHOR INDEX

Aarthi N., 145
Abhijith R.L., 137
Abhishek V., 183
Amrutha Anand, 123
Amrutha M., 163, 235
Anbalagan S., 175
Anchu C.L., 137
Anjana A., 219
Aparna Sureshchandra Kalawate, 215
Ashish Uniyal, 225
Ashmitha Elangovan, 145
Baiju Kochunarayanan, 191, 203
Balaji B.N., 131
Bindu Lakshmanan, 123
Harathi P.B., 145
Harkanwal Singh, 153
Hegde V.D., 231
Indu V Raj, 123
Jebine Jose, 191, 203
Kajal T Annasaheb, 123
Kalesh Sadasivan, 191, 203
Krishna Keerthi K.P., 157
Madhu Thapliyal, 225
Mani Chellappan, 183
Maya George, 203
Mohd Yousuf, 243
Mohsin Ikram, 243
Muhamed Jafer Palot, 191, 203
Pandit, 131
Rabiya Irshad, 243
Ramesha B., 157
Ranjith M.T, 183
Remia K.M., 231
Ryan A. St Laurent, 239
Sajeesh P.K., 157
Sapna Jacob, 239
Seeja Thomachan, 183
Shanas Sudheer, 203
Sheena A., 137
Shivanna B., 131
Sojan Munnar, 191
Sreekumar K.M., 157
Sujitha Prabhakaran Chandrika, 191
Surendragopal K., 183
Syed Faizan Anayat, 243
Tahmeena Maqsood, 243
Teji K.T., 219
Thresiamma Varghese, 229
Toms Augustine, 191, 203
Unnikrishnan M.P., 239
Veena Maurya, 225
Vidya C., 157
Vijayan S., 175
Vijayasree V., 137
Vijaykumar L., 131
Vimala K John, 163, 235
Vinayan Padmanabhan Nair, 191, 203
Viswanath T.K., 231
Yashika Negi, 225

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I, Dr K. D. Prathapan, Secretary, Association for Advancement of Entomology, here by declare that the particulars given above are true to the best of my knowledge and belief.

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